UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO: CIÊNCIAS EM GASTROENTEROLOGIA E HEPATOLOGIA

Polimorfismos da região promotora do *gene* CCR5 em Lúpus Eritematoso Sistêmico

AMANDA HENRIQUE DE OLIVEIRA

DISSERTAÇÃO DE MESTRADO

Porto Alegre

2023

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO: CIÊNCIAS EM GASTROENTEROLOGIA E HEPATOLOGIA

Polimorfismos da região promotora do *gene* CCR5 em Lúpus Eritematoso Sistêmico

AMANDA HENRIQUE DE OLIVEIRA

Dissertação de Mestrado apresentada ao Programa de Pós-Graduação: Ciências em Gastroenterologia e Hepatologia, da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Mestre em Ciências em Gastroenterologia e Hepatologia.

Orientador: Dr. José Artur Bogo Chies

Coorientadora: Dra. Natália Schneider Nunes

Porto Alegre

2023

CIP - Catalogação na Publicação

DE OLIVEIRA, AMANDA HENRIQUE
Polimorfismos da região promotora do gene CCR5 em Lúpus Eritematoso Sistêmico / AMANDA HENRIQUE DE OLIVEIRA. -- 2023.
62 f.
Orientador: José Artur Bogo Chies.

Coorientadora: Natália Schneider Nunes.

Dissertação (Mestrado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências em Gastroenterologia e Hepatologia, Porto Alegre, BR-RS, 2023.

1. CCR5. 2. polimorfismos do promotor CCR5. 3. Lúpus Sistêmico. 4. Lúpus Eritematoso. I. Bogo Chies, José Artur, orient. II. Schneider Nunes, Natália, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

Agradecimentos

Primeiramente agradeço a Deus por me permitir chegar até aqui e estar realizando esse sonho. Agradeço aos meus pais que seguiram incansavelmente me acompanhando ao longo de todo o processo, me acalmando, me incentivando, aplaudindo cada conquista e me reerguendo em cada tombo, minha maior motivação será vocês, sempre. Sou imensamente grata ao meu amor, Victor Hugo que desde o início está comigo, me aconselhando, sendo telespectador de apresentações, puxando a orelha pra estudar e não desanimar, sem você tudo seria mais difícil. Agradeço também a minha irmã, que sempre torceu e esteve do meu lado, me acalmando e mostrando que no fim tudo ia dar certo e deu, tu és um dos pilares que me sustenta, não estaria aqui sem ti. Queria agradecer duas pessoas em especial, minhas amigas Ally e Dé, que sempre escutam meus medos e seguiram sempre presente e me dando força para conseguir mais essa conquista, amo vocês.

Não menos importante e extremamente especial, agradeço ao meu orientador Prof. Dr. José Chies, ele aceitou me orientar no meio de uma tempestade de situações do meu mestrado e logo depois uma pandemia mundial, não foi fácil, mas conseguimos, te agradeço imensamente por me ajudar a chegar neste momento. Um obrigada a minha coorientadora Prof.ª. Dra. Natália Schneider, tudo começou comigo assistindo uma apresentação tua em um evento do mestrado e nisso estamos construindo essa parceria e amizade, obrigada Natty.

Um mega obrigada a todo pessoal do PPG, em especial a Cláudia que nesse período me ajudou muito em todas situações adversas que surgiram, e também agradeço a coordenação por entenderem todas as vezes minha situação e pedidos de prorrogação. Agradeço também, a todos mestres, colegas, amigos, familiares que me acompanharam e torceram por mim, que entenderam minha ausência tantas vezes, o amor, encorajamento e apoio de todos foram meus combustíveis para não desistir, e um obrigada em especial a Kelly, Jordan, Elisa, Bruno, vocês são colegas adoráveis e saber que sobrevivemos ao mestrado é para se comemorar.

"Lembre-se: Você é do tamanho dos seus sonhos."

Roberto Shinyashiki

SUMÁRIO

RESUMO..6

ABSTRACT 8

LISTA DE FIGURAS E TABELAS...11

1 INTRODUÇÃO 12

2 REVISÃO DA LITERATURA 13

2.1. LÚPUS ERITEMATOSO SISTÊMICO (LES) 13

2.1.2 Diagnóstico 14

2.1.3 Tratamento 15

2.2. O que são polimorfismos? 16
2.2.1. CCR5 E SEUS POLIMORFISMOS 18

2.2.2 CCR5 e LES..20

2.2.3 CCR5Δ32 22

4. QUESTÕES DE PESQUISA 25

5. HIPÓTESE 25

6. OBJETIVOS 25

6.1. Objetivo Geral 25

6.2. Objetivos Específicos 25

7. ARTIGO EM INGLÊS 26

8. CONCLUSÕES 53

9. CONSIDERAÇÕES FINAIS 55

REFERENCIAS 56

RESUMO

Introdução: Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune inflamatória crônica e complexa caracterizada por atividade anormal das células B e T [1, 2], produção de autoanticorpos e deposição de imunocomplexos, levando a dano tecidual. Vários estudos têm abordado o papel das quimiocinas e seus receptores em LES, porém não há consenso quanto ao seu envolvimento na patogênese da doença. Entretanto, Mamtani *et al* descreveram a importante associação dos haplótipos humanos da região promotora do CCR5 (HH) HHE e HHG*2 com o risco de desenvolvimento de LES, enquanto HHF*1 foi indicado como possível protetor da mesma [17]. **Objetivo:** O presente trabalho visa investigar o possível papel de polimorfismos da região promotora do gene CCR5 no desenvolvimento e desfecho de LES através da comparação dos genótipos e haplótipos de pacientes vs controles pareados etnicamente. Além disso, será investigada uma variante do gene CCR2 fortemente associada ao gene CCR5. **Métodos e Resultados:** Este estudo incluiu 382 pacientes com LES (289 classificados como de origem europeia e 93 de origem africana) e 375 controles (243 de origem europeia e 132 de origem africana) genotipados para CCR2-64I G>A (rs1799864), CCR5-59353 C> T (rs1799988), CCR5-59356 C>T (rs41469351), CCR5-59402 A>G (rs1800023) e CCR5-59653 C>T (rs1800024) polimorfismos através de PCR-RFLP e sequenciamento direto. Dados anteriores de CCR5Δ32 foram incluídos no estudo para inferir os haplótipos e como um possível fator de confusão na regressão logística binária. Nossos resultados indicaram que CCR5Δ32 e as frequências reduzidas do haplótipo HHG*2 em pacientes de origem europeia estavam associados ao desenvolvimento da doença (p=0,001; OR 3,5; 95% CI 1,6-7,5 e 2,0%,vs. 7,2% residual p= 2,9E-5, respectivamente). Em pacientes de origem africana, as frequências dos haplótipos HHA/HHB, HHC e HHG*2 diferiram entre pacientes e controles (10% versus 20,5%, residual p= 0,003; 29,4%vs. 17,4%, residual p=0,003 e 3,9%vs. 0,8%, residual p=0,023; respectivamente). Considerando as manifestações clínicas da doença, a presença de CCR5Δ32 foi confirmada como fator de suscetibilidade para nefrite classe IV no grupo de origem africana e quando os pacientes foram considerados em conjunto (p corrigido=0,012; OR 3,0; 95% CI 3,0-333,3 e p corrigido= 0,0006; OR 6.8; 95%IC 1,9-2,48, respectivamente). **Conclusão**: Este estudo indica que os polimorfismos da região promotora do gene CCR5 são importantes modificadores da doença em LES. Os dados atuais reforçam o polimorfismo CCR5Δ32 como fator protetor para o desenvolvimento da doença em pacientes Euro-descendentes e como fator de suscetibilidade para nefrite classe IV em pacientes Afro-descendentes. Além disso, também descrevemos uma frequência reduzida de HHA/HHB e uma frequência aumentada de haplótipos HHC e HHG*2 em nossos pacientes Afro-descendentes, o que potencialmente reflete a expressão aumentada de CCR5 em subconjuntos de células específicos e a reduzida expressão de CCR5 em geral.

Palavras-chaves: CCR5; polimorfismos do promotor CCR5; Lúpus Sistêmico; Lúpus eritematoso.

ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic and complex inflammatory autoimmune disease characterized by abnormal activity of B and T cells [1, 2], production of autoantibodies and deposition of immune complexes, leading to tissue damage. Several studies have addressed the role of chemokines and their receptors in SLE, but there is no consensus as to their involvement in the pathogenesis of the disease. Mamtani *et al* described the important association of human haplotypes of the CCR5 promoter regions (HH) HHE and HHG*2 to the risk of SLE development, while HHF*1 was indicated as a possible protective factor [17]. **Objective**: The present work aims to investigate the possible role of polymorphisms from the CCR5 promoter region in SLE development and clinical outcome, by comparing the frequencies of genotypes and haplotypes from patients vs ethnically matched controls. Furthermore, a CCR2 gene variation that is strongly associated to CCR5 will be analyzed. **Methods and results:** This study included 382 patients with SLE (289 classified as of European ancestry and 93 of African ancestry) and 375 controls (243 of European ancestry and 132 of African ancestry) genotyped for CCR2-64I G>A (rs1799864), CCR5-59353 C>T (rs1799988), CCR5-59356 C>T (rs41469351), CCR5-59402 A>G (rs1800023) and CCR5-59653 C>T (rs1800024) polymorphisms by PCR-RFLP and direct sequencing. Previous CCR5Δ32 data was included in the study to infer haplotypes and as a possible confounder in binary logistic regression. Our results indicated that CCR5Δ32 and reduced frequencies of the HHG*2 haplotype in patients of European ancestry were associated to disease development (p=0.001; OR 3.5; 95% CI 1.6-7, 5 and 2.0%, vs. 7.2% residual p= 2.9E-5, respectively). In patients of African descent, the frequencies of HHA/HHB, HHC and HHG*2 haplotypes differed between patients and controls (10% vs. 20.5%, residual p= 0.003; 29.4% vs. 17.4%, residual p =0.003 and 3.9% vs. 0.8%, residual p=0.023, respectively). Considering the clinical manifestations of the disease, the presence of CCR5Δ32 was confirmed as a susceptibility factor for class IV nephritis in the group of African ancestry, or when all patient data was combined (corrected p=0.012; OR 3.0; 95% CI 3, 0-333.3 and corrected p=0.0006; OR 6.8; 95%CI 1.9-2.48, respectively). **Conclusions:** This study indicates that CCR5 promoter polymorphisms are important disease modifiers in SLE. Current data reinforce the CCR5Δ32 polymorphism as a protective factor for the development of the disease in patients of European ancestry and as a susceptibility factor for class IV nephritis in patients of African ancestry. Furthermore, we also describe a reduced frequency of HHA/HHB and an increased frequency of HHC and HHG*2 haplotypes in our patients of African ancestry, which could potentially reflect in a higher expression of CCR5 in specific cell subsets and in a lower expression of overall CCR5.

Keywords: CCR5; CCR5 promoter polymorphisms; Lupus, Systemic; Lupus erythematosus.

LISTA DE ABREVIATURAS

LES	Lúpus Eritematoso Sistêmico
DC	Células Dendríticas
DNA	Ácido desoxirribonucleico
OR	*Odds ratio* (razão de chances)
mRNA	RNA mensageiro
HIV	Vírus da imunodeficiência humana
MCP-1	Proteína quimioatraente de monócitos-1
RM	Ressonância Magnética
MIP-1β	Proteína inflamatória de macrófagos beta
(AINH)	Anti-inflamatório não esteroides
ERKs	Quinases reguladas por sinais extracelulares
CCR5	Receptor quimiocina C-C tipo 5
CCR2	Receptor de quimiocina C-C tipo 2

LISTA DE FIGURAS E TABELAS

ARTIGO

Table 1 – Clinical and laboratory features in Europa-derived, African-derived and in the whole group of SLE patients…………………………………….…….……..33

Table 2 – Genotypes of the CCR5 promoter region polymorphisms and frequency of the variant allele in Europan-derived, African-derived and in the whole group of SLE patients……………………………………………………..…………………34

Table 3 – Binary Logistic Regression comparisons of the presence or absence of the polymorphic variants of CCR5 promoter region polymorphisms in patients and controls as a whole group and by ethnicity – final model…………………………………..35

Table 4 – Groups that presented significant linkage disequilibrium between polymorphisms…………………………………………………………………….……36

Table 5 – Haplotype frequencies and comparisons between patients and controls in European and African-derived groups……………………………,…………38

1 INTRODUÇÃO

Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune inflamatória crônica e complexa caracterizada por atividade anormal das células B e T [1, 2], produção de autoanticorpos e deposição de imunocomplexos, levando a dano tecidual. As quimiocinas e seus receptores são atores centrais na regulação da quimiotaxia de leucócitos na inflamação e acredita-se que tenham um papel importante na patogênese de doenças autoimunes, incluindo LES [3, 4].

Vários estudos têm abordado o papel das quimiocinas e seus receptores em LES, porém não há consenso quanto ao seu envolvimento na patogênese da doença. Não existe um tratamento específico para LES, pois não existe um protocolo padrão para todos os pacientes. Desta forma, uma ampla variedade de medicamentos e normas é empregada, visando uma boa qualidade de vida [5].

Dada a falta de consenso sobre o papel dos receptores de quimiocinas na patogênese de LES e a necessidade de mais estudos nessa área, o presente trabalho visa investigar o possível papel dos polimorfismos da região promotora do gene *CCR5* no desenvolvimento de LES, comparando as frequências dos genótipos e haplótipos com controles pareados etnicamente e analisar se há possível envolvimento dos polimorfismos no desfecho clínico da doença.

2 REVISÃO DA LITERATURA

2.1. LÚPUS ERITEMATOSO SISTÊMICO (LES)

Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune inflamatória crônica e complexa caracterizada por atividade anormal das células B e T [1, 2], produção de autoanticorpos e deposição de imunocomplexos, levando a dano tecidual.

Fatores genéticos e ambientais (incluindo imunológicos e hormonais) são conhecidos por participar na etiologia de LES, porém, a sua causa subjacente exata permanece desconhecida. Vários fatores influenciam a patogênese de LES, como defeitos na apoptose, alterações no sistema complemento e função anormal das células T e B, incluindo alterações no balanço de citocinas ou quimiocinas [2, 6, 7]. As quimiocinas e seus receptores são atores centrais na regulação da quimiotaxia de leucócitos na inflamação e acredita-se que tenham um papel importante na patogênese de doenças autoimunes, incluindo LES [3,4].

Vários estudos têm abordado o papel das quimiocinas e seus receptores em LES, porém não há consenso quanto ao seu envolvimento na patogênese da doença. Os receptores de quimiocinas CCR2 e CCR5 e alguns de seus ligantes (por exemplo, MCP-1, MIP-1β e RANTES) já foram sugeridos como tendo um papel na patogênese de LES [3, 4, 8, 9]. CCR2 e CCR5 são importantes receptores da família das quimiocinas CC, e os genes que codificam estas moléculas estão localizados em um cluster no cromossomo 3 na região 3p21.3-p24 [10]. CCR2 é expresso em monócitos, macrófagos, células dendríticas derivadas do sangue, células T e B, células Natural Killer e basófilos, sendo seus principais ligantes MCP-

1, 2, 3 e 4, salientando-se alta especificidade para MCP-1 [11, 12]. CCR5 é expresso em monócitos, macrófagos, células dendríticas, células T e B, e seus principais ligantes são MIP1β, MIP1α e RANTES [13, 14].

2.1.2 Diagnóstico

Para a realização do diagnóstico de LES consideram-se os critérios clínicos, os quais são manifestados ao longo do tempo e análises laboratoriais. O que dificulta este diagnóstico é a ampla diversidade de sintomatologia, especialmente na fase inicial. A doença se manifesta principalmente com o aparecimento de lesões cutâneas em mulheres jovens nos seus períodos férteis (lembrando-se que sua ocorrência não é exclusiva ao sexo feminino, mas há uma relação aproximada de 9:1 de casos em relação ao sexo masculino) [15]. O *American College of Rheumatology* estabeleceu originalmente onze critérios no diagnóstico de LES e o paciente precisa apresentar quatro para poder ser considerado positivo. Os diferentes critérios usados no diagnóstico são eritema malar, lesão discoide, fotossensibilidade, úlceras orais/nasais, artrite, serosite, comprometimento renal, alterações neurológicas, alterações hematológicas, alterações imunologícas e anticorpos antinucleares. Embora seja raro, alguns pacientes com LES podem não apresentar quatro dos onze critérios citados [16]. No entanto, uma nova visão considera que exames clínicos associados à ocorrência de sintomas imunológicos já são suficientes para o diagnóstico de LES. A Ressonância Magnética (RM) é um dos exames mais indicados em pacientes com alterações neuropsiquiátricas, possibilitando um diagnóstico precoce e tratamento adequado. Os mecanismos patológicos das lesões cerebrais nestes pacientes ainda não estão claros e a visualização por RM torna-se fundamental para o estudo dos mesmos [17]. Adicionalmente, autoanticorpos são as evidências mais importantes nos exames laboratoriais para o diagnóstico do LES. Os fatores nucleares (FAN) estão presentes em quase todos os casos de doença ativa e reagem com componentes do núcleo celular. Apesar de não serem específicos para LES, nenhuma outra situação fisiológica ou de doença apresenta frequência e títulos tão elevados destes componentes [5].

As manifestações clínicas da doença incluem apresentações renais, hematológicas, imunológicas, neurológicas, cutâneas, cardiovasculares e artrite. A doença afeta principalmente mulheres em idade reprodutiva, e como anteriormente mencionado, numa frequência em torno de 9 mulheres para 1 homem. No Brasil, existem poucos dados sobre a incidência de LES. Os dados disponíveis indicam que a incidência varia de 4,8/100.000 habitantes/ano [18] a 8,7/100.000 habitantes/ano no Nordeste [19].

2.1.3 Tratamento

Não existe um tratamento específico para LES, pois não se tem um protocolo padrão para todos os pacientes. Desta forma, uma série de medidas, entre medicamentos e normas, são empregadas para que se proporcione uma boa qualidade de vida aos pacientes [5].

De acordo com SATO *et al.* (2002), algumas medidas gerais devem ser adotadas como parte importante da abordagem terapêutica, tais como: oferecer informações gerais sobre a doença, o seu diagnóstico e tratamento, fornecer acompanhamento psicológico e incentivar a prática de atividade física regular (exceto em períodos de atividade sistêmica da doença).

Além disso, é de fundamental importância a indicação de uma dieta balanceada, evitando excesso de sal, carboidrato e lipídios. Os pacientes devem ser aconselhados a evitar a exposição prolongada ao sol e a prática do tabagismo. O tratamento medicamentoso é prescrito de acordo com as manifestações clínicas e a gravidade da doença. Anti-inflamatórios não esteroides (AINH) são utilizados no tratamento de artralgias, artrite, febre e serosite, pericardite e pleurites leves. Os antimaláricos também são indicados e os corticosteróides são utilizados para manifestações mais graves ou quando os medicamentos mencionados acima não forem eficazes [16, 17, 20, 21]. A dosagem destes medicamentos deve ser controlada para minimizar os efeitos colaterais.

Antimaláricos, como a cloroquina e o difosfato de cloroquina, são indicados para reduzir a atividade da doença e com isso evitar uso de corticosteroides [20, 21]. Os antimaláricos são essenciais e devem ser administrados por pelo menos seis meses após o controle dos sintomas da doença. Corticosteroides como a prednisona são administrados nas fases mais agudas da doença [16, 17]. A melhoria dos sintomas geralmente é notada após vários dias de tratamento. Na maioria dos pacientes, os antiinflamatórios são suficientes para controlar a doença [20, 22].

2.2. O que são polimorfismos?

Polimorfismos (originário do grego, poli= muitas, morfismos= formas) genéticos são variações na sequência da molécula de DNA, podendo ser resultantes de trocas, repetições, inserções ou deleções de uma ou mais bases nitrogenadas.

Estas alterações podem estar localizadas em várias regiões do gene: região promotora, codificadora (éxons) ou não codificadora (íntrons) [23, 71].

Para ser considerado um alelo, uma dada alteração ou polimorfismo deve ter frequência maior que 1% em uma dada população, percentual este que a diferencia de uma mutação pontual da molécula de DNA ainda não caracterizada como polimórfica, a qual apresenta, portanto, frequência inferior a 1%. O genoma humano possui um número estimado de 30.000 e 35.000 genes e a sequência do DNA apresenta similaridade de 99,9% entre diferentes indivíduos [71].

Esta incrivelmente pequena diferença entre os indivíduos corresponde à presença dos polimorfismos. Os polimorfismos na região promotora e codificadora têm maior probabilidade de modificar o funcionamento do gene e, consequentemente, a função da proteína formada [23]. O alelo é uma das diversas variantes de um gene em uma determinada região cromossômica, conhecida por locus (do latim “lugar”).

O locus é o local fixo no cromossomo onde está localizado determinado gene. O genótipo representa o conjunto de alelos que ocupam um determinado locus em um indivíduo, recebendo a denominação de homozigoto quando a mesma variante ocupa o locus nos dois cromossomos homólogos ou heterozigoto quando duas variantes diferentes ocupam o locus em questão [24].

Um polimorfismo pode ter sua atividade dependente de heterozigose ou homozigose, sendo mais expressivo em casos de homozigose para alelo de risco. Vale ressaltar que um polimorfismo não é necessariamente deletério, podendo inclusive estar relacionado a um benefício para o portador do alelo variante [72].

Sendo assim, estudos que envolvem a relação dos polimorfismos à susceptibilidade de situações de risco podem auxiliar o desenvolvimento de melhores estratégias terapêuticas, incluindo modificações de estilo de vida como prevenção de doenças [25]. Por exemplo, estudos no âmbito da nutrigenômica e nutrigenética abordam estratégias nutricionais eficientes para silenciar genes que codificam condições de agravo a doenças. Este tipo de relação gene vs doença demonstra a importância de estudos dos fatores genéticos, os quais podem servir de base para novas terapias e estratégias de prevenção [73].

2.2.1. CCR5 E SEUS POLIMORFISMOS

As quimiocinas são moléculas reguladoras fundamentais no desenvolvimento, diferenciação e migração de leucócitos [26, 27, 28, 29 30]. O gene CCR5 e a molécula por ele codificada são bastante conhecidos devido ao seu papel clássico como molécula co-receptora utilizada pelos vírus HIV [26,31, 32, 33, 34, 35, 36]. O receptor de quimiocina CC tipo 5 (CCR5) interage principalmente com as quimiocinas CCL3 (MIP-1α), CCL4 (MIP-1β) e CCL5 (RANTES), as quais atuam como agonistas de CCR5, estimulando a migração celular e mediando respostas inflamatórias. Adicionalmente, a quimiocina MCP-3/CCL7 é o principal ligante antagonista do CCR5 [37, 38, 39, 40].

Além disso, CCR5 atua nas respostas imunes Th1 e a falta de sinalização por meio desta molécula leva a um direcionamento para respostas Th2. A proteína CCR5 tem um efeito regulador nas células inflamatórias, sendo que a expressão de CCR5 já foi observada em alguns subconjuntos de células T reguladoras (Treg). [41,42, 43]. A proteína CCR5 humana (352 resíduos) é codificada pelo gene *CCR5* [Cromossomo 3 (3p.21.31)], que se apresenta altamente polimórfico [37, 44]. É fundamental considerar que CCR5 medeia diferentes funções imunológicas, sendo assim possível que a ausência ou alteração de sua expressão promova alterações de difícil detecção, mas com significado médico em um ambiente ou contexto específico.

O caminho do CCR5 entre a membrana plasmática e o meio intracelular é mediado por diferentes moléculas, incluindo clatrinas, β-arrestina 2 e quinase regulada por sinal extracelular (ERK) 1 [45, 46]. Além disso, o CD4 intracelular regula a expressão de CCR5 na superfície celular [47]. Dentre os polimorfismos do CCR5, o CCR5Δ32 (rs333) tem sido intensamente estudado em diferentes populações humanas. A frequência de CCR5Δ32 é bastante variável, dependendo da origem étnica da população [52, 74].

Em geral, a frequência do alelo Δ32 é alta em populações de origem europeia (por exemplo, 16% na Noruega e 11% na Alemanha) e baixa ou ausente em populações africanas e asiáticas [48]. No entanto, há exceções devido a eventos migratórios. Por exemplo, a frequência do alelo Δ32 é relativamente alta em certos grupos populacionais da África do Sul (13%) e no Chile (12%) [48]. Além disso, a frequência do alelo Δ32 pode ser bastante variável dentro do mesmo país. No Brasil, a frequência do alelo na população geral está em torno de 4–5% [48, 49]. Na região sul do país, a frequência pode chegar a 9% devido à migração de populações europeias para esta região [50, 51, 52].

Em geral, considera-se que a ausência de CCR5 observada em indivíduos homozigotos da variante CCR5Δ32 não está associada a qualquer alteração fisiológica essencial grave. Embora seja geralmente aceito que indivíduos homozigotos dessa variante não apresentam deficiências imunológicas ou clínicas graves, [35,53] respostas inflamatórias exacerbadas já foram relatadas em associação com a variante CCR5Δ32 [54].

2.2.2 CCR5Δ32

CCR5Δ32 é a variante genética do gene CCR5 mais estudada devido ao seu forte efeito protetor contra a infecção pelo HIV. Esta variante consiste em uma deleção de 32 pares de bases na região codificadora de CCR5, o que causa uma mudança do quadro de leitura e resulta em uma proteína truncada que não é direcionada para a superfície celular. Indivíduos com genótipo heterozigoto para CCR5Δ32, se infectados pelo HIV, têm uma pequena proteção contra a progressão da doença devido à expressão reduzida de CCR5 na superfície das células T $CD4^+$. Já indivíduos homozigotos para esse polimorfismo (Δ32/Δ32) apresentam proteção virtualmente total contra a infecção pelo HIV tipo 1, uma vez que nenhuma expressão de CCR5 é presente na superfície celular e, portanto, o vírus não tem onde ligar-se para promover uma efetiva invasão celular. [62, 63, 64, 65, 66, 67, 68, 69].

No Brasil, dois estudos avaliaram a variante CCR5Δ32 em lúpus. Schauren *et al.* investigaram o papel do CCR5Δ32 em pacientes com LES e controles saudáveis do Rio Grande do Sul. Uma frequência menor do alelo CCR5Δ32 foi encontrada em pacientes euro-brasileiros (2,7%) quando comparados aos controles euro-brasileiros (7,5%), sugerindo um papel protetor da variante contra o desenvolvimento de lúpus eritematoso sistêmico [52]. No entanto, pacientes com o alelo CCR5Δ32 apresentaram maior predisposição ao desenvolvimento de nefrite classe IV em comparação aos pacientes sem o alelo, o que sugere um resultado clínico mais grave associado à variante genética [52].

Baltus *et al.* avaliaram as frequências de CCR5Δ32 em pacientes e controles no estado do Paraná. Ao contrário do primeiro estudo, a frequência do alelo CCR5Δ32 foi estatisticamente maior em pacientes (6,8%) do que em controles saudáveis (1,9%), sugerindo a variante como um fator de risco para LES. Além disso, ao estratificar a amostra de acordo com a etnia, os pesquisadores identificaram que indivíduos euro-brasileiros portadores do CCR5Δ32 eram mais propensos a desenvolver LES do que os pacientes afro-brasileiros portadores da variante [70]. Cabe salientar que CCR5Δ32 é uma variante pleiotrópica, podendo portanto promover resultados diferentes em diferentes situações.

2.2.3 CCR5 e LES

O progresso da doença é afetado pelo início de respostas imunes inatas e adquiridas, enquanto é acelerado por repetidas respostas imunes excessivas, as quais levam a danos teciduais por meio de reações autoimunes e deposição de complexos imunes [55, 56]. A inflamação crônica e as respostas imunes hiperativas em pacientes com LES desempenham um papel determinante na patogênese da doença. Vários tipos de células imunes contribuem para o priming, incidência, progressão e desfecho da doença no LES. As células dendríticas (DCs), como células imunes inatas centrais, são células primárias que determinam a direção das respostas imunes à tolerância ou ao estado inflamatório em pacientes com lúpus [57,58]. Os receptores de quimiocinas, como os receptores de superfície celular imune, direcionam o recrutamento celular e a migração para os linfonodos primários, induzindo assim respostas inflamatórias ou regulatórias [75].

A expressão de CCR5 (ligantes CCL3, CCL4 e CCL5), CCR4 (ligantes CCL17 e CCL22) e CCR3 (ligantes eotaxina e eotaxina-2) em diferentes células imunes está associada ao progresso da inflamação em LES [59, 60]. A expressão de CCR5 em células T ativadas está associada a maior infiltração celular e inflamação.

O papel do CCR5 na patogênese de LES ainda é controverso, porém, Mamtani *et. al* descreveram uma importante associação dos haplótipos humanos da região promotora do CCR5 (HH) HHE e HHG*2 com o risco de desenvolver LES e uma tendência do HHF*1 à proteção da mesma [4]. Além disso, Aguilar *et. al*. sugeriram um leve aumento na produção de autoanticorpos, no desenvolvimento de nefrite e na gravidade da doença em pacientes portadores do alelo CCR5Δ32 [61]. De acordo com o trabalho publicado pelo nosso grupo, Schauren *et. al*, mulheres de origem europeia com LES apresentaram menor frequência do alelo CCR5Δ32 quando comparadas a controles pareados etnicamente, enquanto a presença do alelo foi associada a nefrite grave em pacientes de origem africana [52].

3.JUSTIFICATIVA

Com o melhor entendimento do envolvimento dos polimorfismos da região promotora de CCR5 no desenvolvimento de LES, será possível aprimorar o prognóstico e opções de tratamento da doença, melhorando o desfecho clínico e qualidade de vida dos pacientes.

4. QUESTÕES DE PESQUISA

Quais fatores relacionados ao perfil imunogenético do hospedeiro (especialmente considerando-se os genes *CCR2* e *CCR5*) influenciam o desenvolvimento de LES, seu estabelecimento e progressão da doença?

5. HIPÓTESE

O perfil imunogenético indicará como o indivíduo responderá à doença, seja em relação ao tratamento, progressão ou remissão da doença.

6. OBJETIVOS

6.1. Objetivo Geral

Avaliar a influência de polimorfismos da região promotora de CCR5 em indivíduos com LES.

6.2. Objetivos Específicos

– Compreender a interação entre a imunogenética em população normal e LES;

– Analisar a possível influência e suscetibilidade à LES nos indivíduos com polimorfismo na região promotora de CCR5;

7. ARTIGO EM INGLÊS

CCR5 PROMOTER REGION POLYMORPHISMS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Juliana da Silveira Schauren[1], Amanda Henrique de Oliveira[3], Camila Rosat Consiglio[1], Odirlei André Monticielo[2], Ricardo Machado Xavier[2], João Carlos Tavares Brenol[2], Natália Schneider Nunes[3,4], José Artur Bogo Chies[1]

Immunogenetics Laboratory, Genetics Department , Biosciences Institute, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre – Brazil[1]

Rheumatology Division, Internal Medicine Department, Hospital de Clinicas de Porto Alegre – HCPA/UFRGS, Porto Alegre - Brazil[2]

Gastroenterology and Hepatology Sciences Graduate Program, UFRGS, Porto Alegre, RS, Brazil[3]

Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States[4]

Corresponding author: José Artur Bogo Chies.
Department of Genetics, Institute of Biosciences, UFRGS, Caixa Postal 15053.

CEP 91501-970, Porto Alegre, RS, Brazil.

Fax: +55-51-3308-7311/ Email: jabchies@terra.com.br

Manuscript to be submitted to "International Journal of Immunogenetics"

CCR5 PROMOTER REGION POLYMORPHISMS IN SYSTEMIC LUPUS ERYTHEMATOSUS

Juliana da Silveira Schauren[1], Amanda Henrique de Oliveira[3], Camila Rosat Consiglio[1], Odirlei André Monticielo[2], Ricardo Machado Xavier[2], João Carlos Tavares Brenol[2], Natália Schneider Nunes[3,4], José Artur Bogo Chies[1]

Immunogenetics Laboratory, Genetics Department , Biosciences Institute, Universidade Federal do Rio Grande do Sul – UFRGS, Porto Alegre – Brazil[1]

Rheumatology Division, Internal Medicine Department, Hospital de Clinicas de Porto Alegre – HCPA/UFRGS, Porto Alegre - Brazil[2]

Gastroenterology and Hepatology Sciences Graduate Program, UFRGS, Porto Alegre, RS, Brazil[3]

Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, United States[4]

ABSTRACT

Given the lack of consensus for the role of chemokine receptors in Systemic Lupus Erythematosus (SLE) pathogenesis, there's a need for further investigation. Therefore, this study aims to explore the role of CCR5 promoter region polymorphisms in the development of SLE. Using the comparison of the genotypes and haplotypes from patients with SLE vs ethnically matched controls, the association of the polymorphisms in the clinical outcome of the disease was investigated. This study included 382 SLE patients (289 European-derived and 93 African-derived) and 375 controls (243 European-derived and 132 African-derived) genotyped for the CCR2-64I G>A (rs1799864), CCR5-59353 C>T (rs1799988), CCR5-59356 C>T (rs41469351), CCR5-59402 A>G (rs1800023) and CCR5-59653 C>T (rs1800024) polymorphisms though PCR-RFLP and direct sequencing. Previous data from CCR5Δ32 analyzes were included in the study to infer the haplotypes and as a possible confounding factor in the binary logistic regression. Our results indicated that European-derived patients presented reduced frequencies of CCR5Δ32 and the HHG*2 haplotype in comparison to controls, which was associated development of the disease (p=0.001; OR 3.5; 95%CI 1.6-7.5 and 2.0%, vs. 7.2% residual p= 2.9E-5, respectively). Additionally, the HHA/HHB, HHC and HHG*2 haplotype frequencies differed between African-derived patients and controls (10% vs. 20.5%, residual p= 0.003; 29.4% vs. 17.4%, residual p=0.003 and 3.9% vs. 0.8%, residual p=0.023; respectively). Considering the clinical manifestations of the disease, CCR5Δ32 presence was confirmed as a susceptibility factor to class IV nephritis in the African-derived group and when all patients were grouped for comparison (pcorrected=0.012; OR 3.0; 95%CI 3.0-333.3 and pcorrected= 0.0006; OR 6.8; 95%CI 1.9-2.48, respectively). In conclusion, this study indicates that CCR5 promoter polymorphisms are important disease modifiers in SLE. Present data reinforces CCR5Δ32 polymorphism as a protective factor for the development of the disease in European-derived patients and as a susceptibility factor for class IV nephritis in African-derived patients. Furthermore, we also describe a reduced frequency of HHA/HHB and an enhanced frequency of HHC and HHG*2 haplotypes in African-derived patients, which could reflect in a higher expression of CCR5 in specific cell subsets and in a reduced expression of CCR5 overall.

Keywords: CCR5, CCR5 promoter polymorphisms, Lupus, Systemic Lupus Erythematosus.

1. INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease characterized by abnormal activity of B and T cells [1, 2], autoantibodies production and immunocomplex deposition leading to tissue damage. The clinical manifestation of the disease include renal, hematological, immunological, neurological, cutaneous, and cardiovascular alterations, in addition to arthritis. The disease affects mainly women in reproductive age, in a frequency of 9 women to 1 man. In Brazil, there are few data regarding the incidence of SLE. Available data indicate that the disease incidence ranges from 4.8/100,000 inhabitants/year [3] to 8.7/100,000 inhabitants/year in the northeast [4]. The disease seems to be more frequent in African-derived individuals and less frequent in native Africans [5, 6].

Genetic, immunological and environmental factors are known to participate in the etiology of the disease, where many components play a role its pathogenesis; those include defects in apoptosis, alterations of the complement system, abnormal function of T and B cells, and alterations in the cytokines or chemokines balance [2, 7-14]. Chemokines and their receptors are central players in the regulation of leucocytes chemotaxis in inflammation, and they are thought to have an important role in the pathogenesis of autoimmune diseases, including SLE [15-17].

Several studies have addressed the role of chemokines and their receptors in SLE, however, there is no consensus regarding their involvement in the pathogenesis of the disease. For instance, the chemokine receptors CCR2 and CCR5 and their ligands MCP-1, MIP-1β and RANTES have been previously suggested to have a role in SLE pathogenesis [13, 15, 17-20]. CCR2 and CCR5 are important chemokine receptors of the CC chemokine family, localized in a cluster on chromosome 3 in the 3p21.3-p24 region [21]. CCR2 is expressed on monocytes, macrophages, blood-derived dendritic cells, T and B cells, Natural Killer cells and basophils, and its main ligands are MCP-1, 2, 3 and 4, with high specificity for MCP-1 [22, 23]. CCR5 is expressed on monocytes, macrophages, dendritic cells, T and B cells, and its main ligands are MIP1β, MIP1α e RANTES [24-27].

CCR5 role in SLE pathogenesis is still controversial. Mamtani *et al.* has described the association of the CCR5 promoter region human haplotypes (HH) HHE and HHG*2 to the risk of developing SLE, while HHF*1 was described as a protective factor [17]. Additionally, Aguilar *et al.* suggested a slight increase in the production of autoantibodies, nephritis development and disease severity in patients bearing the CCR5Δ32 allele [28]. According to the previous work from our group [60], SLE European-derived women presented a lower frequency of the CCR5Δ32 allele when compared to ethnically matched controls, and the presence of the allele was associated to severe nephritis in African-derived patients.

Given the lack of consensus for the role of chemokine receptors in SLE pathogenesis, this study aims to investigate the role of the CCR5 promoter region polymorphisms in the development of SLE through the comparison of patients' genotypes and haplotypes to ethnically matched controls, analyzing the involvement of the polymorphisms in the clinical outcome of the disease.

2. METHODS

2.1. Study populations

There were included 382 SLE patients (289 European-derived and 93 African-derived) from the state of Rio Grande do Sul, in the southern region of Brazil. Records and samples were provided by the Rheumatology Clinic of the "Hospital de Clínicas de Porto Alegre" (HCPA). The control group included 375 healthy blood donors (243 European-derived and 132 African-derived); of these, 301 from Rio Grande do Sul (RS) and 74 from Rio de Janeiro (RJ), in the southeast region of Brazil. Both patients and controls were recruited by convenience sampling. Ethnical classification was based on the physical appearance as judged by the researcher at the time of blood collection and on data about the ethnicity of parents/grandparents reported by the participants.

The self-reported "color" classification frequently used in Brazil is well documented in different studies [29-31], including previous studies from our group [32, 33]. It is noteworthy that, in Southern Brazilian populations from the same region addressed in this study, the individuals classified as "White" have a mean European ancestry of 85.5% and ~1% of African ancestry, while individuals classified as "Brown" and "Black" have approximately 45% of African, 44% of European and 11% of Amerindian ancestry [29]. This study also revealed that the genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected.

SLE patients were diagnosed according to the revised American College of Rheumatology criteria [34, 35]. The clinical and laboratory variables evaluated in this study were accessed at diagnosis and updated according to the medical records of the patients and included: photosensitivity, malar rash, discoid rash, oral or nasal ulcers, arthritis, serositis -pleuritis or -pericarditis, nephritis and neurological disease - seizures or psychosis. The laboratory evaluation included the presence of hematological disorders such as hemolytic anemia, leukopenia, lymphopenia or thrombocytopenia, immunological disorders, and positive antinuclear antibody (ANA). SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) [36] and SLICC (Systemic Lupus International Collaborating Clinics) damage index [37] were applied to each patient as a measurement of disease activity and cumulative damage, respectively. Lupus nephritis classification was performed according to the World Health Organization (WHO) classification from 1982, revised in 2003 [38], and renal biopsy recommendation criteria were persistent protein excretion greater than 500 mg/day and/or an active urinary sediment with hematuria, typically dysmorphic, and cellular casts [39]. Class IV nephritis was included in the analyses of clinical manifestations, as our previous work indicates that CCR5Δ32 African-derived carriers presented a higher predisposition to this class of nephritis.The present study was approved by the ethics committee from the HCPA/UFRGS and informed consent was obtained from all subjects.

2.2. PCR and Genotyping

DNA samples were extracted by salting out method and stored at -20°. The CCR5 promoter region polymorphisms CCR5-59353 C>T (rs1799988), CCR5-59356 C>T (rs41469351), CCR5-59402 A>G (rs1800023) and CCR5-59653 C>T (rs1800024) were amplified in each sample by Polymerase Chain Reaction (PCR) using the primers LK84 and LK87 previously described [40, 41]. Amplification was performed in a final volume of 30 µl containing 5 mM each of dNTP; 37.5 mM MgCl2; 5 pmol/µl of each primer; 1 unit of Platinum Taq DNA Polymerase in 10X PCR-specific buffer (Invitrogen-Life Technologies, São Paulo, Brazil) and 10 to 100 ng of genomic DNA. The initial denaturation cycle was carried out at 94ºC for 5 minutes; followed by 35 cycles at 95ºC for 30s; 60ºC for 30 s; 72ºC for 1 minute and by a final extension step at 72ºC for 5 minutes. The amplification product was quantified (10 to 50 ng/µl) by Low Mass (Invitrogen-Life Technologies, São Paulo, Brazil) in 1% agarose gel. Each PCR product was purified and sequenced using LK84 primer in an ABI 3730 XL DNA Sequencer according to the manufacturer's manual by Macrogen Korea (Macrogen Inc.). Genotyping was performed by interpretation of chromatogram peaks through FinchTV Software Version 1.4.0. The CCR2-64I G>A (rs1799864) polymorphism was genotyped through PCRRFLP (Restriction Fragment Length Polymorphism) assay using BsaBI restriction endonuclease as defined by Smith *et al.* [42] and the assay was performed as described by Vargas *et al.* [43].

2.3. Statistical analysis

Due to the lack of some clinical and/or laboratory data in the medical records, some analysis may not include all the individuals genotyped in this work. The genotypic frequencies of the polymorphisms in the promoter region of CCR5, including CCR2, were compared with Hardy-Weinberg expectations using chi-square tests. Comparisons of the polymorphisms' frequencies by ethnicity in control and patient groups were performed using Binary Logistic Regression controlling for gender. For susceptibility analysis, we considered the presence or absence of the polymorphic variant designated as carriers and non-carriers, respectively.

These frequencies were compared between patients and controls, between control groups from RS and RJ and between patients with or without a given clinical or laboratory feature using Binary Logistic Regression controlling for possible confounding factors. Ranks for SLICC and SLEDAI were analyzed by Mann-Whitney tests. Statistical analysis was performed using the software SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and WinPepi [44]. Haplotypes were estimated by Mlocus software and included the polymorphisms CCR2-64I, CCR5-59353, CCR5-59356, CCR5-59402, CCR5-59653 and CCR5Δ32. Of note, CCR5Δ32 genotypes were previously obtained by our group (Schauren *et al.* 2013). Haplotype frequencies were compared between patients and controls in European and African-derived groups by chi-square tests.

Significance level was established as $p=0.05$ (two-tailed) and Bonferroni correction for multiple comparisons was applied when the p value was significant.

3. RESULTS

3.1. Patients and controls

Due to the known differences in the disease incidence [5] and in the CCR5 promoter region genotypic frequencies [45] among different ethnicities, analyzes were stratified or controlled by the most frequent ethnicities in our population, European and African ancestry. Patients comprised of 350 women and 32 men. The average age of patients was 45.4 ± 15.1 years old and the mean age at diagnosis was 32.6 ± 13.9 years old. The median value for the SLEDAI accessed at the first evaluation was 1.0 (0 and 4.0 for percentiles 25 and 75, respectively) and the SLICC index was 1.0 (0 and 2.0). Other clinical characteristics are shown in Table 1. Comparisons between European-derived and African-derived patients concerning disease manifestations have already been addressed in this cohort by Monticielo *et al.* [46]. Comparisons indicated that European-derived patients presented higher photosensitivity and reduced presence of leukopenia/lymphopenia and the autoantibodies anti-Ro/SS-A and anti-La/SS-B [46].

Furthermore, it was observed that male patients presented a higher frequency of nephritis than female patients, although the number of male patients in this group is relatively small. Control group consisted of 90 women and 260 men, and the mean age of controls was 41.4 ±10.1 years old. All analyzes involving control group were controlled by this factor, age. Comparing the genotypic frequencies of all polymorphisms addressed in this study, controlling for gender and age, control groups from RS and RJ did not differ statistically. Therefore, we joined these groups and considered the control group as one.

Table 1: *Clinical and laboratory features in European-derived, African-derived and in the whole group of SLE patients:*

Patients' features	European-derived N/total N (%)	African-derived N/total N (%)	Whole N/Total N (%)
Females	263/289 (91.0)	87/93 (93.5)	350/382 (91.6)
Age (years)	45.5 ± 15.0	44.7 ± 13.5	45.3 ± 14.7
Age at diagnosis (years)	32.1 ± 13.8	33.7 ± 13.7	32.6 ± 13.9
SLEDAI[a*]	1.0 (0, 4.0)	1.0 (0, 4.0)	1.0 (0, 4.0)
SLICC[b*]	1.0 (0, 2.0)	1.0 (0, 2.0)	1.0 (0, 2.0)
Malar rash	164/289 (56.7)	48/93 (51.6)	214/384 (55.7)
Discoid rash	43/289 (14.9)	15/93 (16.1)	58/384 (15.1)
Photosensitivity	234/289 (81.0)	55/93 (59.1)	290/384 (75.5)
Oral/nasal ulcers	107/289 (37.0)	30/93 (32.3)	137/384 (35.7)
Arthritis	240/289 (83.0)	75/93 (80.6)	315/384 (82.0)
Serositis	83/287 (28.9)	34/93 (36.6)	117/382 (31.6)
Neurologic disorders	35/289 (12.1)	10/93 (10.8)	46/384 (12.0)
Nephritis	124/289 (42.9)	43/93 (46.2)	167/384 (43.5)
Hematological disorders	216/189 (74.7)	82/93 (88.2)	300/384 (78.1)
Immunological disorders	196/286 (68.5)	64/93 (68.8)	261/381 (68.5)
ANA[c]	287/288 (99.7)	92/93 (98.9)	381/383 (98.2)

[a] Systemic Lupus Erythematosus Disease Activity Index, [b] Systemic Lupus International Collaborating Clinics and [c] Antinuclear Antibody. * Median, percentiles 25.75. (total numbers can vary due to the lack of data).

3.2. Genotypes

Genotypic frequencies of the polymorphisms in patients and controls are presented in table 2. All groups were in Hardy-Weinberg equilibrium, exception made for CCR5-59653 frequencies in European-derived control group and in the whole group (data not shown). We observed that in the control group, the frequencies of the CCR5-59353, CCR5-59356 and CCR5-59402 polymorphisms were different between European- and African-derived groups (pcorrected=0.018, pcorrected=0.004 and pcorrected<0.001, respectively). In SLE group, only the CCR5-59356 polymorphism frequency differed between European- and African-derived groups (pcorrected=0.002).

Table 2: *Genotypes of the CCR5 promoter region polymorphisms and frequency of the variant allele in European-derived, African-derived and in the whole group of SLE patients*:

Polymorphism	Genotypes	European-derived Patients	European-derived Controls	African-derived Patients	African-derived Controls	Whole Patients	Whole Controls
CCR2-641	**wt/wt (%)**	217 (75.9)	194 (80.5)	72 (77.4)	65 (70.6)	293 (76.1)	259 (77.8)
	wt/641 (%)	65 (22.7)	42 (17.4)	18 (22.7)	26 (17.4)	83 (21.6)	68 (20.4
	641/641 (%)	4 (1.4)	5 (2.1)	3 (1.4)	1 (2.1)	9 (2.3)	6 (1.8)
	Total n	286	241	93	92	385	333
	641(frequency)	0.13	0.11	0.13	0.15	0.13	0.12
CCR5-59353	**CC (%)**	82 (28.6)	74 (31.1)	26 (28.3)	32 (24.4)	113 (29.4)	106 (28.7)
	CT (%)	152 (53.0)	116 (48.7)	43 (46.7)	67 (51.2)	195 (50.6)	183 (49.6)
	TT (%)	53 (18.5)	48 (20.2)	23 (25.0)	32 (24.4)	77 (20.0)	80 (21.7)
	Total n	287	238	92	131	385	369
	T (frequency)	0.45	0.45	0.48	0.50	0.45	0.46
CCR5-59356	**CC (%)**	281 (97.6)	234 (98.3)	76 (83.5)	100 (76.4)	363 (94.3)	334 (90.5)
	CT (%)	7 (7.24)	4 (1.7)	14 (15.4)	29 (22.1)	21 (5.5)	33 (8.9)
	TT (%)	0 (0)	0 (0)	1 (1.1)	2 (1.5)	1 (0.3)	2 (0.5)
	Total n	288	238	91	131	385	369
	T (frequency)	0.01	0.01	0.08	0.13	0.03	0.05
CCR5-59402	**AA (%)**	116 (40.3)	104 (43.7)	49 (53.3)	87 (66.4)	170 (44.0)	191 (51.8)
	AG (%)	144 (50.0)	103 (43.3)	32 (34.8)	43 (32.8)	177 (45.9)	146 (39.6)
	GG (%)	28 (9.7)	31 (13.0)	11 (12.0)	1 (0.8)	39 (10.1)	32 (8.7)
	Total n	288	238	92	131	386	369
	G (frequency)	0.35	0.35	0.29	0.17	0.33	0.28
CCR5-59402	**CC (%)**	220 (76.4)	194(81.5)*	70 (76.1)	87 (66.4)	294 (76.2)	281 (76.2)*
	CT (%)	63 (21.9)	38 (16.0)	19 (20.7)	37 (28.3)	82 (21.2)	75 (20.3)*
	TT (%)	5 (1.7)	6 (2.5)	3 (3.3)	7 (5.3)	10 (2.6)	13 (3.5)
	Total n	288	238	92	131	386	369
	T (frequency)	0.13	0.10	0.14	0.19	0.13	0.14

* Not in Hardy-Weinberg equilibrium.

3.3. Susceptibility analysis

As our previous work indicates CCR5Δ32 (32 bp deletion which generates a truncated protein that does not reach the cell surface) as a protective factor to the development of SLE, we have included this polymorphism, ethnicity, gender and potential interactions as possible confounding factors in the binary logistic regression analysis. The final model included CCR2-64I, CCR5-59353, CCR5-59356, CCR5-

59402, CCR5-59653 and CCR5Δ32 polymorphisms, gender, and ethnicity. In the analysis considering patient and control groups as a whole, the genotypic frequencies of the CCR2-64I, CCR5-59353, CCR5-59356, CCR5-59402 and CCR5-59653 did not differ between these groups, while only female gender and the CCR5Δ32 wild type homozygous (wt/wt) were associated with the disease, as shown in table 3 ($p<0.001$; OR 25; 95%CI 15.8-39.4 and $p=0.010$; OR 2.5; 95%CI 1.2-5.0, respectively). When split by ethnicity, the analysis of European-derived groups also indicated female gender and CCR5Δ32 wt/wt association with the disease ($p<0.001$; OR 22; 95%CI 13.3-36.4 and $p=0.001$; OR 3.5; 95%CI 1.6-7.5, respectively). Meanwhile, in the African-derived group, only female gender was associated with the disease ($p<0.001$; OR 58.4; 95%CI 16.8-203.2) (table 3).

Table 3: *Binary Logistic Regression comparisons of the presence or absence of the polymorphic variants of CCR5 promoter region polymorphisms in patients and controls as a whole group and by ethnicity – final model.*

Patients vs. controls	Variables	OR*	95% CI	Significance
Whole (371 vs. 285)	**CCR2 (wt/wt[c])**	0.6	0.1-2.2	0.420
	CCR5-59353 (CC)	1.3	0.7-2.6	0.452
	CCR5-59356 (CC)	0.9	0.4-2.7	0.992
	CCR5-59402 (AA)	0.8	0.4-1.5	0.540
	CCR5-59653 (CC)	1.4	0.4-5.5	0.581
	CCR5Δ32 (wt/wt)	2.5	1.2-5.0	0.030*
	Gender (female)	25.0	15.8-39.4	<<0.001*
	Ethnicity (European)	0.7	0.4-1.2	0.158
European-derived (282 vs. 234)	**CCR2 (wt/wt[c])**	0.8	0.2-3.4	0.752
	CCR5-59353 (CC)	1.0	0.5-2.3	0.899
	CCR5-59356 (CC)	0.7	0.1-3.7	0.698
	CCR5-59402 (AA)	1.0	0.5-2.0	0.935
	CCR5-59653 (CC)	1.1	0.2-4.8	0.923
	CCR5Δ32 (wt/wt)	3.5	1.6-7.5	0.003*
	Gender (female)	22.0	13.3-36.4	<<0.001*
African-derived (89 vs. 51)	**CCR2 (wt/wt[c])**	0.2	0.01-2.9	0.236
	CCR5-59353 (CC)	2.1	0.4-10.0	0.340
	CCR5-59356 (CC)	0.8	0.2-3.7	0.813
	CCR5-59402 (AA)	0.5	0.1-1.8	0.277
	CCR5-59653 (CC)	2.8	0.2-35.3	0.425
	CCR5Δ32 (wt/wt)	0.1	0.01-1.5	0.105
	Gender (female)	58.4	16.8-203.2	<<0.001*

[a] OR: Odds Ratio, [b] CI: confidence Interval and [c] wt/wt: Homozygous for wild type allele.
*Bonferroni corrected *p* values.

3.4. Clinical manifestations

The prevalence of the clinical and laboratory features of SLE patients was compared considering the presence or absence of each variant allele, while controlling for confounding factors. Considering patient group as a whole and splitting the analysis by ethnicity, only the CCR5Δ32 presence was confirmed as a susceptibility factor to class IV nephritis in the African-derived group, and when the patient group was considered as a whole (pcorrected=0.012; OR 3.0; 95%CI 3.0-333.3 and pcorrected= 0.0006; OR 6.8; 95%CI 1.9-2.48, respectively). European-derived patients showed the same tendency, however, after correction, the analysis lost its significance. None of the clinical and laboratory features included in the analyzes were significantly associated to any of the other polymorphic variants.

3.5. Haplotypes

Linkage disequilibrium (LD) between polymorphisms for patients and controls are presented in table 4. LD was considered significant when $p<0.05$ and Lewontin Normalized Coefficient D'=1. None of the polymorphisms in LD presented complete LD ($r^2<0.50$). All groups presented LD between the CCR5-59353 and CCR5-59356, which was expected due to the vicinity of these polymorphisms (3bp distance). Beyond the data presented in table 4, we observed that in the LD analysis between the CCR5-59353, CCR5-59356, CCR5-59402 and CCR5-59653 polymorphisms, almost all combinations had a D'>0.9.

Table 4: *Groups that presented significant linkage disequilibrium between polymorphisms:*

Polymorphisms	CCR2-64I	CCR5-59353	CCR5-59356	CCR5-59402	CCR5-59653
CCR2-64I	-	-	-	-	-
CCR5-59353	CAD	-	-	-	-
CCR5-59356	C, CAD	C, CED, CAD, L, LED, LAD	-	-	-
CCR5-59402	CAD	CAD, LAD	C, CAD, L, LAD	-	-
CCR5-59653		CED, CAD, LAD	CAD	C, CED, CAD, LAD	-
CCR5Δ32		LAD			C, CED

C: Control group as a whole, CED: European-derived control group, CAD: African-derived control group, L: Lupus patient group as a whole, LED: Lupus European-derived patient group, LAD: Lupus African-derived patient group.

A set of seven evolutionary distinct human haplotypes in the promoter region of the CCR5 gene has already been described, namely HHA, HHB, HHC, HHD, HHE, HHF (F*1, F*2), and HHG (G*1, G*2) [47]. Since the frequencies of other haplotypes were very low, we joined rare haplotypes into two groups considering the presence or absence of the CCR5Δ32 allele: rare bearing d32 and rare not bearing d32.

Haplotype frequencies and comparisons between patients and controls in European- and African-derived groups are presented in table 5. Comparisons between patients and controls haplotype frequencies showed that overall frequencies differed in both European- and African-derived groups (pcorrected = 0.006 and 0.002, respectively). In the European-derived group, only the HHG*2 haplotype frequency was significantly higher in controls than in patients (7.2% vs. 2.0%, residual p= 2.9E-5). In the African-derived group, the HHA/HHB, HHC and HHG*2 haplotype frequencies differed between patient and control groups (10% vs. 20.5%, residual p= 0.003; 29.4% vs. 17.4%, residual p=0.003 and 3.9% vs. 0.8%, residual n p=0.023; respectively).

Table 5: *Haplotype frequencies and comparisons between patients and controls in European- and African-derived groups:*

HAPLOTYPES				European-derived Patients	European-derived Controls	African-derived Patients	African-derived Controls
HHA or HHB	**V T C A C**	wt	(%)	51 (9.1)	44 (9.3)	**18 (10.0)[b]**	**53 (20.5)[b]**
HHC	V T C G C	wt	(%)	192 (34.2)	161 (34.1)	**53 (29.4)[c]**	**45 (17.4)[c]**
HHD	V T T A C	wt	(%)	7 (1.2)	4 (0.8)	16 (8.9)	33 (12.7)
HHE or HHG*1	V C C A C	wt	(%)	229 (40.8)	176 (37.3)	60 (33.3)	75 (29.0)
HHF*1	V C C A T	wt	(%)	3 (0.5)	2 (0.4)	2 (1.1)	8 (3.1)
HHF*2	I C C A T	wt	(%)	67 (11.9)	48 (10.2)	23 (12.8)	42 (16.2)
HHG*2	V C C A C	d32	(%)	**11 (2.0)[a]**	**34 (7.2)[a]**	**7 (3.9)[d]**	**2 (0.8)[d]**
Rre bearing d32	- - - - -	d32	(%)	4 (0.7)	1 (0.2)	0 (0)	0 (0)
Rare not bearing d32	- - - - -	wt	(%)	8 (1.4)	2 (0.4)	1 (0.6)	1 (0.4)
Total				572	472	180	259
Significance (*p*corrected)				**0.006**		**0.002**	

[a] residual p=2.9E-5, [b] residual p= 0.003, [c] residual p=0.003 and [d] residual p=0.023.

4. DISCUSSION

In Brazil, two studies have evaluated the CCR5Δ32 variant in lupus. Schauren *et al.* investigated the role of CCR5Δ32 in healthy controlled patients from Rio Grande do Sul. A lower frequency of the CCR5Δ32 allele was found in Euro-Brazilian patients (2.7%) compared to Euro-Brazilian controls (7.5%), suggesting a variant protective role against the development of systemic lupus erythematosus. This work corroborates our previous findings on the involvement of CCR5Δ32 in SLE protection in patients of European origin, and susceptibility to class IV nephritis in patients of African origin and in SLE patients as a whole. [60]

In the previous study, European-derived patients presented a lower frequency of the CCR5Δ32 polymorphism (in other words, a higher frequency of homozygous for the wild type allele, which promotes a normal production of this cell surface receptor) when compared to ethnically matched controls, suggesting that the lack of

CCR5 in the immune cells surface could be a protective factor against the disease [60].

In the present study, CCR5Δ32 polymorphism was included in the logistic regression model and, even when controlled for gender and the CCR5 promoter region polymorphisms, including CCR2-64I, it was the only polymorphism significantly associated with protection against the disease. Furthermore, HHG*2 haplotype, bearing the CCR5Δ32 allele, was the only haplotype associated to SLE protection in European-derived patients.

These findings are interestingly opposed to the results found by Mamtani *et al.*, which indicated that the HHG*2 haplotype was a risk factor for developing SLE [17], probably reflecting different genetic background of the studied populations. Interestingly, a recent study performed in Germany indicated that $CCR5^{+}/CCR3^{+}$ T helper cells ratio was enhanced in patients with active Cutaneous Lupus Erythematosus [48], which is in accordance to our findings. Moreover, another German study demonstrated that the presence of the CCR5Δ32 has a protective effect on the development of another autoimmune disease, Rheumatoid arthritis (RA) [49], which was also associated with a diminished expression of the CCR5Δ32 allele in European patients overall [50].

In the African-derived group analysis, the frequencies of the haplotypes HHA/HHB, HHC and HHG*2 differed between patient and control groups. HHG*2 haplotype frequency was slightly increased in patients when compared to controls, which was the opposite of European-derived patients, and corroborates the results of Mamtani *et al.* [17].

This finding is very interesting, since HHG*2 bears the CCR5Δ32 allele. This allele is not originally present in native African populations [51], and its presence in

African-derived individuals results from miscegenation. None of the studies regarding CCR5Δ32 involvement in SLE addressed African-derived patients [17, 28, 52]. Picton *et al.* described that CCR5 promoter haplotypes influence CCR5 expression in Natural Killer (NK) and in T cell subsets in South African populations [53]. They demonstrated that HHC^+ haplotype increased the expression of CCR5 in NK cells (independent of the presence of CCR5Δ32) and in $CD8^+$ T cells (dependent on the presence of CCR5Δ32) in South African Caucasian individuals, and that HHA haplotype was associated with a diminished expression of CCR5 in $CD8^+$ T cells.

Bearing this in mind, we can infer that the diminished frequency of HHA and the enhanced frequency of HHC in our African-derived patients could be related to a higher expression of CCR5 in specific cell subsets and to a lower expression of CCR5 overall.

Regarding CCR2-64I polymorphism, no direct association with SLE was found, in accordance to Aguilar *et al.* [28]. Data available in the literature is conflicting. The study conducted by Amoura *et al.* indicated that $CD4^+CCR2^+$ T cells are decreased in French patients during lupus flares [54], however the study conducted by Li *et al.* indicates that CCR2 expression is higher in Chinese patients with active disease [16]. The same study reported increased CCR5 mRNA levels in SLE patients compared to controls, which was consistent to the findings of Al-Saleh *et al.* They have reported a significantly elevated expression of CCR5 and CXCR3 on the surface of $CD4^+$ T cells of active SLE patients vs SLE patients in remission and healthy controls [55].

Concerning clinical manifestations, only the CCR5Δ32 was confirmed as a susceptibility factor to class IV nephritis, as previously discussed [60]. That was also true when controlling for the presence of the CCR5 promoter region polymorphisms, including CC2-64I, gender and ethnic origin.

The apparent controversial role of CCR5 described by the present study is not a novelty in autoimmune diseases. This subject has been extensively debated in RA [50, 56, 57]. It is suggested that the reduced expression of CCR5 is protective against the development of RA, and our analyzes in SLE follow the same trend.

Baltus *et al.* evaluated CCR5Δ32 frequencies in patients and controls in the state of Paraná, Brazil. The frequency of the CCR5Δ32 allele was statistically higher in patients (6.8%) vs controls (1.9%), suggesting the variant as a risk factor for SLE. Furthermore, when stratifying the sample according to ethnicity, researchers identified Euro-Brazilian individuals carrying CCR5Δ32 as more likely to develop SLE than African-Brazilian patients carrying the variant. However, CCR5Δ32 is a pleiotropic variant, promoting different results in different situations [61].

The study also states that the CCR5Δ32 polymorphism may be associated with genetic susceptibility to SLE and early age at onset of the disease, suggesting it as one of the influential factors in the development and prognosis of SLE. The literature shows the need for further studies on the influence of CCR5 and other polymorphisms of chemokine receptors on the immune response in patients with SLE [61]. The identification of the genotypes of these chemokine receptors as biomarkers of the development and/or severity of autoimmune diseases, such as SLE, may provide new therapeutic options considering the individual's genetic profile [61].

In an overall assessment, reduced CCR5 expression appears to be protective against the development of SLE. However, the ethnic origin is an extremely important factor related to SLE and CCR5 expression, and should receive special attention when interpreting the data. Additionally, the influence of CCR5 and CCR5∆32 in different diseases should not be generalized. The role of these two factors is quite variable in different conditions and may change at different timepoints of the same disease. For instance, both CCR5 and CCR5Δ32 affect the development of osteomyelitis, whereas in Chagas disease, CCR5 is involved in the pathogenesis of the disease and the CCR5Δ32 variant has little influence. Yet, In schistosomiasis, the action of CCR5 seems to be based on regulatory mechanisms [62].

Additionally, some of the studies have small sample sizes, which can result in biased conclusions. Furthermore, he unclear role of CCR5 may be due to the presence of this receptor in cells with different functions in the immune system. For instance, CCR5 presence on the surface of T helper cells promotes their migration towards target tissues and exacerbates inflammation, whereas T regulatory cells bearing CCR5 promote a tolerogenic environment and reduction of inflammation. CCR5 may also contribute to the resolution of inflammation by acting as a decoy receptor [58, 59].

In conclusion, this study indicates that CCR5 promoter polymorphisms are important disease modifiers in SLE, and that genetic background should be carefully considered when investigating the association of SLE and chemokine receptors. Present data reinforces CCR5Δ32 polymorphism as a protective factor for the development of the disease in European-derived patients, and as a susceptibility factor for class IV nephritis in African-derived patients. Furthermore, we demonstrate a reduced frequency of HHA/HHB and an enhanced frequency of HHC and HHG*2 in our African-derived patients, which could be related to a higher expression of CCR5 in specific cell subsets and a lower expression of CCR5 overall. These findings are further evidence that CCR5 promoter polymorphisms are important in autoimmune diseases and emphasize the need for more studies in this area.

REFERENCES

1. Dörner, T., Giesecke, C., Lipsky, P.E. 2011. Mechanisms of B cell autoimmunity in SLE. Arthritis research & therapy, 13: 243.

2. Moulton, V.R., Tsokos, G.C. 2011. Abnormalities of T cell signaling in systemic lupus erythematosus. Arthritis research & therapy, 13: 207.

3. Nakashima, C.A., Galhardo, A.P., Silva, J.F., Fiorenzano, G.R., Santos, A.B., Leite, M.F., Nogueira, M.A., Menolli, P.V., Menolli, R.A. 2011. Incidence and clinical-laboratory aspects of systemic lupus erythematosus in a Southern brazilian city. Rev Bras Reumatol, 51: 231-9.

4. Vilar, M.J., Sato, E.I. 2002. Estimating the incidence of systemic lúpus erythematosus in a tropical region (Natal, Brazil). Lupus, 11: 528-32.

5. McCarty, D.J., Manzi, S., Medsger, T.A., Jr., Ramsey-Goldman, R., LaPorte, R.E., Kwoh, C.K. 1995. Incidence of systemic lupus erythematosus. Race and gender differences. Arthritis Rheum, 38: 1260-70.

6. Borchers, A.T., Naguwa, S.M., Shoenfeld, Y., Gershwin, M.E. 2010. The geoepidemiology of systemic lupus erythematosus. Autoimmunity reviews, 9: A277-87.

7. Munoz, L.E., Lauber, K., Schiller, M., Manfredi, A.A., Herrmann, M. 2010. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Ver Rheumatol, 6: 280-9.

8. Shao, W.-H., Cohen, P.L. 2011. Disturbances of apoptotic cell clearance in systemic lupus erythematosus. Arthritis research & therapy, 13: 202.

9. Gullstrand, B., Martensson, U., Sturfelt, G., Bengtsson, A.A., Truedsson, L. 2009. Complement classical pathway components are all important in clearance of apoptotic and secondary necrotic cells. Clin Exp Immunol, 156: 303-11.

10. Truedsson, L., Bengtsson, A.A., Sturfelt, G. 2007. Complement deficiencies and systemic lupus erythematosus. Autoimmunity, 40: 560-6.

11. Jacob, N., Stohl, W. 2010. Autoantibody-dependent and autoantibody-independent roles for B cells in systemic lupus erythematosus: past, present, and future. Autoimmunity, 43: 84-97.

12. Wong, C.K., Lit, L.C., Tam, L.S., Li, E.K., Wong, P.T., Lam, C.W. 2008. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity. Clin Immunol, 127: 385-93.

13. Lit, L.C., Wong, C.K., Tam, L.S., Li, E.K., Lam, C.W. 2006. Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. Ann Rheum Dis, 65: 209-15.

14. Schiffer, L., Kumpers, P., Davalos-Misslitz, A.M., Haubitz, M., Haller, H., Anders, H.J., Witte, T., Schiffer, M. 2009. B-cell-attracting chemokine CXCL13 as a marker of disease activity and renal involvement in systemic lupus erythematosus (SLE). Nephrol Dial Transplant, 24: 3708-12.

15. Eriksson, C., Eneslatt, K., Ivanoff, J., Rantapaa-Dahlqvist, S., Sundqvist, K.G. 2003. Abnormal expression of chemokine receptors on T-cells from patients with systemic lupus erythematosus. Lupus, 12: 766-74.

16. Li, Y.-m., Chen, Z.-q., Yao, X., Yang, A.-z., Li, A.-s., Liu, D.-m., Gong, J.-q. 2010. mRNA Expression of Chemokine Receptors on Peripheral Blood Mononuclear Cells and Correlation with Clinical Features in Systemic Lupus Erythematosus Patients. Chinese Medical Sciences Journal, 25: 162-168.

17. Mamtani, M., Rovin, B., Brey, R., Camargo, J.F., Kulkarni, H., Herrera, M., Correa, P., Holliday, S., Anaya, J.-M., Ahuja, S.K. 2008. CCL3L1 gene-containing segmental duplications and polymorphisms in CCR5 affect risk of systemic lúpus erythaematosus. Annals of the rheumatic diseases, 67: 1076-83.

18. Lu, M.M., Wang, J., Pan, H.F., Chen, G.M., Li, J., Cen, H., Feng, C.C., Ye, D.Q. 2012. Increased serum RANTES in patients with systemic lupus erythematosus. Rheumatol Int, 32: 1231-3.

19. Kaneko, H., Ogasawara, H., Naito, T., Akimoto, H., Lee, S., Hishikawa, T., Sekigawa, I., Tokano, Y., Takasaki, Y., Hirose, S.I. et al. 1999. Circulating levels of beta-chemokines in systemic lupus erythematosus. J Rheumatol, 26: 568-73.

20. Lima, G., Soto-Vega, E., Atisha-Fregoso, Y., Sanchez-Guerrero, J., Vallejo, M., Vargas-Alarcon, G., Llorente, L. 2007. MCP-1, RANTES, and SDF-1 polymorphisms in Mexican patients with systemic lupus erythematosus. Hum Immunol, 68: 980-5.

21. Samson, M., Soularue, P., Vassart, G., Parmentier, M. 1996. The genes encoding the human CC-chemokine receptors CC-CKR1 to CC-CKR5 (CMKBR1-CMKBR5) are clustered in the p21.3-p24 region of chromosome 3. Genomics, 36: 522-6.

22. Myers, S.J., Wong, L.M., Charo, I.F. 1995. Signal transduction and ligand specificity of the human monocyte chemoattractant protein-1 receptor in transfected embryonic kidney cells. J Biol Chem, 270: 5786-92.

23. Frade, J.M., Mellado, M., del Real, G., Gutierrez-Ramos, J.C., Lind, P., Martinez, A.C. 1997. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. J Immunol, 159: 5576-84.

24. Combadiere, C., Ahuja, S.K., Tiffany, H.L., Murphy, P.M. 1996. Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1(alpha), MIP-1(beta), and RANTES. J Leukoc Biol, 60: 147-52.

25. Raport, C.J., Gosling, J., Schweickart, V.L., Gray, P.W., Charo, I.F. 1996. Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. J Biol Chem, 271: 17161-6.

26. Samson, M., Labbe, O., Mollereau, C., Vassart, G., Parmentier, M. 1996. Molecular cloning and functional expression of a new human CC-chemokine receptor gene. Biochemistry, 35: 3362-7.

27. Murphy, P.M., Baggiolini, M., Charo, I.F., Hébert, C.a., Horuk, R., Matsushima, K., Miller, L.H., Oppenheim, J.J., Power, C.a. 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacological reviews, 52: 145-76.

28. Aguilar, F., Núñez-Roldán, A., Torres, B., Wichmann, I., Sánchez-Román, J., González-Escribano, M.F. 2003. Chemokine receptor CCR2/CCR5 polymorphism in Spanish patients with systemic lupus erythematosus. The Journal of rheumatology, 30: 1770-4.

29. Pena, S.D.J., Di Pietro, G., Fuchshuber-Moraes, M., Genro, J.P., Hutz, M.H., Kehdy, F.D.S.G., Kohlrausch, F., Magno, L.A.V., Montenegro, R.C., Moraes, M.O. et al. 2011. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PloS one, 6: e17063.

30. Leite, T.K.M., Fonseca, R.M.C., de França, N.M., Parra, E.J., Pereira, R.W. 2011. Genomic ancestry, self-reported "color" and quantitative measures of skin pigmentation in brazilian admixed siblings. PloS one, 6: e27162.

31. Parra, F.C., Amado, R.C., Lambertucci, J.R., Rocha, J., Antunes, C.M., Pena, S.D. 2003. Color and genomic ancestry in Brazilians. Proc Natl Acad Sci U S A, 100: 177-82.

32. Vargas, A.E., Marrero, A.R., Salzano, F.M., Bortolini, M.C., Alegre, P. 2006. Frequency of CCR5 Δ32 in Brazilian populations, 5: 321-325.

33. Veit, T.D., Cordero, E.a.a., Mucenic, T., Monticielo, O.a., Brenol, J.C.T., Xavier, R.M., Delgado-Cañedo, a., Chies, J.a.B. 2009. Association of the HLA-G 14 bp polymorphism with systemic lupus erythematosus. Lupus, 18: 424-30.

34. Tan, E.M., Cohen, A.S., Fries, J.F., Masi, A.T., McShane, D.J., Rothfield, N.F., Schaller, J.G., Talal, N., Winchester, R.J. 1982. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 25: 1271-7.

35. Hochberg, M.C. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum, 40: 1725.

36. Bombardier, C., Gladman, D.D., Urowitz, M.B., Caron, D., Chang, C.H. 1992. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum, 35: 630-40.

37. Gladman, D., Ginzler, E., Goldsmith, C., Fortin, P., Liang, M., Urowitz, M., Bacon, P., Bombardieri, S., Hanly, J., Hay, E. et al. 1996. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum, 39: 363-9.

38. Weening, J.J. 2004. The Classification of Glomerulonephritis in Systemic Lupus Erythematosus Revisited. Journal of the American Society of Nephrology, 15: 241-250.

39. Falk, M.J., Dugan, R.B., O'Riordan, M.A., Matthews, A.L., Robin, N.H. 2003. Medical Geneticists' duty to warn at-risk relatives for genetic disease. American journal of medical genetics. Part A, 120A: 374-80.

40. Kostrikis, L.G., Neumann, a.U., Thomson, B., Korber, B.T., McHardy, P., Karanicolas, R., Deutsch, L., Huang, Y., Lew, J.F., McIntosh, K. et al. 1999. A polymorphism in the regulatory region of the CC-chemokine receptor 5 gene influences perinatal transmission of human immunodeficiency virus type 1 to African-American infants. Journal of virology, 73: 10264-71.

41. Kostrikis, L.G., Huang, Y., Moore, J.P., Wolinsky, S.M., Zhang, L., Guo, Y., Deutsch, L., Phair, J., Neumann, A.U., Ho, D.D. et al. 1998. A chemokine receptor CCR2 allele delays HIV-1 disease progression and is associated with a CCR5 promoter mutation. Nature medicine, 4: 350-353.

42. Smith, M.W., Dean, M., Carrington, M., Winkler, C., Huttley, G.A., Lomb, D.A., Goedert, J.J., O'Brien, T.R., Jacobson, L.P., Kaslow, R. et al. 1997. Contrasting genetic influence of CCR2 and CCR5 variants on HIV-1 infection and disease progression. Hemophilia Growth and Development Study (HGDS), Multicenter AIDS Cohort Study (MACS), Multicenter Hemophilia Cohort Study (MHCS), San Francisco City Cohort (SFCC), ALIVE Study. Science, 277: 959-65.

43. Vargas, a.E., da Silva, M.a.L., Silla, L., Chies, J.a.B. 2005. Polymorphisms of chemokine receptors and eNOS in Brazilian patients with sickle cell disease. Tissue antigens, 66: 683-90.

44. Abramson, J.H. 2004. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiol Perspect Innov, 1: 6.

45. Martinson, J.J., Hong, L., Karanicolas, R., Moore, J.P., Kostrikis, L.G. 2000. Global distribution of the CCR2-64I/CCR5-59653T HIV-1 disease-protective haplotype. AIDS (London, England), 14: 483-9.

46. Monticielo, O.A., Chies, J.A., Mucenic, T., Rucatti, G.G., Junior, J.M., da Silva, G.K., Glesse, N., dos Santos, B.P., Brenol, J.C., Xavier, R.M. 2010. Mannosebinding lectin gene polymorphisms in Brazilian patients with systemic lúpus erythematosus. Lupus, 19: 280-7.

47. Mummidi, S., Bamshad, M., Ahuja, S.S., Gonzalez, E., Feuillet, P.M., Begum, K., Galvis, M.C., Kostecki, V., Valente, a.J., Murthy, K.K. et al. 2000. Evolution of human and non-human primate CC chemokine receptor 5 gene and mRNA. Potential roles for haplotype and mRNA diversity, differential haplotype-specific transcriptional activity, and altered transcription factor binding to polymorphic nucleotides. The Journal of biological chemistry, 275: 18946-61.

48. Freutel, S., Gaffal, E., Zahn, S., Bieber, T., Tuting, T., Wenzel, J. 2011.Enhanced CCR5+/CCR3+ T helper cell ratio in patients with active cutaneous lúpus erythematosus. Lupus, 20: 1300-4.

49. Rossol, M., Pierer, M., Arnold, S., Keysser, G., Burkhardt, H., Baerwald, C., Wagner, U. 2009. Negative association of the chemokine receptor CCR5 d32 polymorphism with systemic inflammatory response, extra-articular symptoms and joint erosion in rheumatoid arthritis. Arthritis research & therapy, 11: R91.

50. Lee, Y.H., Bae, S.C., Song, G.G. 2012. Association between the chemokine receptor 5 delta32 polymorphism and rheumatoid arthritis: a meta-analysis. Mod Rheumatol.

51. Martinson, J.J., Chapman, N.H., Rees, D.C., Liu, Y.T., Clegg, J.B. 1997. Global distribution of the CCR5 gene 32-basepair deletion. Nat Genet, 16: 100-3.

52. Martens, H.a., Gross, S., van der Steege, G., Brouwer, E., Berden, J.H.M., de Sevaux, R., Derksen, R.H.W.M., Voskuyl, A.E., Berger, S.P., Navis, G.J. et al. 2010. Lack of association of C-C chemokine receptor 5 Δ32 deletion status with rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, and disease severity. The Journal of rheumatology, 37: 2226-31.

53. Picton, A.C.P., Paximadis, M., Tiemessen, C.T. 2012. CCR5 promoter haplotypes differentially influence CCR5 expression on natural killer and T cell subsets in ethnically divergent HIV-1 uninfected South African populations. Immunogenetics, 64: 795-806.

54. Amoura, Z., Combadiere, C., Faure, S., Parizot, C., Miyara, M., Raphaël, D., Ghillani, P., Debre, P., Piette, J.-C., Gorochov, G. 2003. Roles of CCR2 and CXCR3 in the T cell-mediated response occurring during lupus flares. Arthritis and rheumatism, 48: 3487-96.

55. Al-Saleh, J., el-Eissawy, S. 2006. The role of T helper cell subsets in athogenesis of Systemic Lupus Erythematosus and their relation to disease activity. Egypt J Immunol, 13: 41-8.

56. Martens, H.A., Kallenberg, C.G., Bijl, M. 2009. Role of CCR5 Delta32 bp deletion in RA and SLE. Autoimmunity, 42: 260-2.

57. Prahalad, S. 2006. Negative association between the chemokine receptor CCR5-Delta32 polymorphism and rheumatoid arthritis: a meta-analysis. Genes Immun, 7: 264-8.

58. Doodes, P.D., Cao, Y., Hamel, K.M., Wang, Y., Rodeghero, R.L., Kobezda, T., Finnegan, A. 2009. CCR5 is involved in resolution of inflammation in proteoglycan-induced arthritis. Arthritis and rheumatism, 60: 2945-53.

59. Dobaczewski, M., Xia, Y., Bujak, M., Gonzalez-Quesada, C., Frangogiannis, N.G. 2010. CCR5 signaling suppresses inflammation and reduces adverse remodeling of the infarcted heart, mediating recruitment of regulatory T cells. The American Journal of Pathology, 176: 2177-87.

60. Schauren JS, Marasca JA, Veit TD, et al. CCR5delta32 in systemic lupus erythematosus: implications for disease susceptibility and outcome in a Brazilian population. Lupus. 2013;22(8):802-809.

61. Baltus THL, Kallaur AP, Lozovoy MAB, Morimoto HK, Delongui F, Alfieri DF, et al.. Ccr5Δ32 (Rs333) Polymorphism is Associated With the Susceptibility to Systemic Lupus Erythematosus in Female Brazilian Patients. Rheumatol Int (2016) 36:7–15. doi: 10.1007/s00296-015-3308-z

62. Ellwanger JH, Kaminski VL, Rodrigues AG, Kulmann-Leal B, Chies JAB. CCR5 and CCR5Δ32 in bacterial and parasitic infections: Thinking chemokine receptors outside the HIV box. *Int J Immunogenet*. 2020;47(3):261-285. doi:10.1111/iji.12485

8. CONCLUSÕES

Concluímos que o haplótipo HHG*2, portando o alelo CCR5Δ 32, foi o único haplótipo associado à proteção de LES em pacientes. Nas análises de grupos de ancestralidade africana, as frequências dos haplótipos HHA/HHB, HHC e HHG*2 diferiram quando comparados ao grupo controle. A frequência do haplótipo HHG*2 foi aumentada em pacientes de ancestralidade africana, enquanto o oposto foi encontrado em pacientes de ancestralidade europeia. Este alelo não está originalmente presente em populações africanas nativas, e sua presença em indivíduos de ancestralidade africana resulta da miscigenação. Tendo isso em mente, podemos inferir que a frequência diminuída de HHA e a frequência aumentada de HHC em nossos pacientes de ancestralidade africana podem estar relacionadas a uma expressão aumentada de CCR5 em subconjuntos de células específicas e a uma expressão reduzida de CCR5 em geral.

O papel controverso de CCR5 descrito neste trabalho não é uma novidade em doenças autoimunes. Entretanto, LES parece seguir a mesma tendência observada em Artrite Reumatoide, sendo que em uma avaliação global, a expressão reduzida de CCR5 parece ser protetora contra o desenvolvimento de LES. A origem étnica é um fator extremamente importante relacionada ao desenvolvimento de LES e à expressão de CCR5, e deve receber atenção especial. Além disso, o papel controverso de CCR5 pode ser devido à presença deste receptor em células com diferentes funções no sistema imunológico.

De acordo com nossos resultados, em pacientes de ancestralidade europeia, CCR5Δ32 e as frequências reduzidas do haplótipo HHG*2 estão associados ao desenvolvimento da doença (p=0,001; OU 3,5; 95% CI 1,6-7,5 e 2,0%,vs. 7,2% residual p= 2,9E-5, respectivamente). Em pacientes de ancestralidade africana, as frequências dos haplótipos HHA/HHB, HHC e HHG*2 diferiram entre pacientes e controles (10% vs 20,5%, residual p= 0,003; 29,4%vs. 17,4%, residual p=0,003 e 3,9%vs. 0,8%, residual p=0,023; respectivamente). Considerando as manifestações clínicas da doença, a presença de CCR5Δ32 foi confirmada como fator de suscetibilidade para nefrite classe IV no grupo de ancestralidade africana e quando os pacientes foram considerados em conjunto (p corrigido=0,012; OU 3,0; 95% CI 3,0-333,3 e p corrigido= 0,0006; OU 6.8; 95%IC 1,9-2,48, respectivamente). Em conclusão, descrevemos uma frequência reduzida de HHA/HHB e uma frequência aumentada de haplótipos HHC e HHG*2 em nossos pacientes de ancestralidade africana, o que potencialmente reflete em uma expressão aumentada de CCR5 em subconjuntos de células específicos e em uma expressão reduzida de CCR5 geral. Estudos adicionais devem ser realizado para o melhor entendimento desta resposta imune.

9. CONSIDERAÇÕES FINAIS

Neste trabalho, demonstramos o possível papel de polimorfismos na região promotora do gene CCR5 como modificadores da doença em pacientes com LES. Nossos dados demonstram que a variante CCR5Δ32 atua como fator protetor para o desenvolvimento da doença em pacientes de ancestralidade europeia e como fator de suscetibilidade para nefrite classe IV em pacientes de ancestralidade africana. A literatura atual reforça a necessidade de mais estudos nessa área, visto que há muitos resultados controversos. Desta forma, poderemos também esclarer o real impacto desta variante em diferentes condições genéticas, ambientais, étnicas e clínicas.

REFERENCIAS

1. Dörner, T., Giesecke, C., Lipsky, P.E. 2011. Mechanisms of B cell autoimmunity in SLE. Arthritis research & therapy, 13: 243.

2. Moulton, V.R., Tsokos, G.C. 2011. Abnormalities of T cell signaling in systemic lupus erythematosus. Arthritis research & therapy, 13: 207.

3. . Eriksson, C., Eneslatt, K., Ivanoff, J., Rantapaa-Dahlqvist, S., Sundqvist, K.G. 2003. Abnormal expression of chemokine receptors on T-cells from patients with systemic lupus erythematosus. Lupus, 12: 766-74.

4. Mamtani, M., Rovin, B., Brey, R., Camargo, J.F., Kulkarni, H., Herrera, M., Correa, P., Holliday, S., Anaya, J.-M., Ahuja, S.K. 2008. CCL3L1 gene-containing segmental duplications and polymorphisms in CCR5 affect risk of systemic lúpus erythaematosus. Annals of the rheumatic diseases, 67: 1076-83.

5. OMONTE, R.K.; LENZ, M. A. S.; BATISTA, J. S. Lúpus eritematoso: uma revisão de literatura. Centro Integrado de Saúde Prof. Roberto Elias, 2006.

6. Munoz, L.E., Lauber, K., Schiller, M., Manfredi, A.A., Herrmann, M. 2010. The role of defective clearance of apoptotic cells in systemic autoimmunity. Nat Ver Rheumatol, 6: 280-9.

7. Schiffer, L., Kumpers, P., Davalos-Misslitz, A.M., Haubitz, M., Haller, H., Anders, H.J., Witte, T., Schiffer, M. 2009. B-cell-attracting chemokine CXCL13 as a marker of disease activity and renal involvement in systemic lupus erythematosus (SLE). Nephrol Dial Transplant, 24: 3708-12.

8. Lit, L.C., Wong, C.K., Tam, L.S., Li, E.K., Lam, C.W. 2006. Raised plasma concentration and ex vivo production of inflammatory chemokines in patients with systemic lupus erythematosus. Ann Rheum Dis, 65: 209-15.

9. Lima, G., Soto-Vega, E., Atisha-Fregoso, Y., Sanchez-Guerrero, J., Vallejo, M., Vargas-Alarcon, G., Llorente, L. 2007. MCP-1, RANTES, and SDF-1 polymorphisms in Mexican patients with systemic lupus erythematosus. Hum Immunol, 68: 980-5.

10. Samson, M., Soularue, P., Vassart, G., Parmentier, M. 1996. The genes encoding the human CC-chemokine receptors CC-CKR1 to CC-CKR5 (CMKBR1-CMKBR5) are clustered in the p21.3-p24 region of chromosome 3. Genomics, 36: 522-6.

11. Myers, S.J., Wong, L.M., Charo, I.F. 1995. Signal transduction and ligand specificity of the human monocyte chemoattractant protein-1 receptor in transfected embryonic kidney cells. J Biol Chem, 270: 5786-92.

12. Frade, J.M., Mellado, M., del Real, G., Gutierrez-Ramos, J.C., Lind, P., Martinez, A.C. 1997. Characterization of the CCR2 chemokine receptor: functional CCR2 receptor expression in B cells. J Immunol, 159: 5576-84.

13. Combadiere, C., Ahuja, S.K., Tiffany, H.L., Murphy, P.M. 1996. Cloning and functional expression of CC CKR5, a human monocyte CC chemokine receptor selective for MIP-1(alpha), MIP-1(beta), and RANTES. J Leukoc Biol, 60: 147-52.

14. Murphy, P.M., Baggiolini, M., Charo, I.F., Hébert, C.a., Horuk, R., Matsushima, K., Miller, L.H., Oppenheim, J.J., Power, C.a. 2000. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacological reviews, 52: 145-76.

15. ASSIS, M. R.; BAAKLINI, C. E. Como Diagnosticar e Tratar Lúpus Eritematoso Sistêmico. Revista Brasileira de Medicina, São Paulo, v. 66, n. 9 p. 274-285, 2009

16. SATO. E, I. 2002. Consenso brasileiro para o tratamento do lúpus eritematoso sistêmico (LES). Revista Brasileira de Reumatologia, São Paulo, v. 42, n. 6, p. 362-370, 2002.

17. CARNEIRO. E.; REIS. C.; PINTO. P.; BERNARDES. J, M.; SILVA. M, L, R.; SALGADO. A. Lúpus Eritematoso Sistêmico: A Ressonância magnética no Envolvimento do Sistema Nervoso Central. Acta Médica Portuguesa, Lisboa, v.19, n.2, p.459-465, 2006.

18. Nakashima, C.A., Galhardo, A.P., Silva, J.F., Fiorenzano, G.R., Santos, A.B., Leite, M.F., Nogueira, M.A., Menolli, P.V., Menolli, R.A. 2011. Incidence and clinical-laboratory aspects of systemic lupus erythematosus in a Southern brazilian city. Rev Bras Reumatol, 51: 231-9.

19. Vilar, M.J., Sato, E.I. 2002. Estimating the incidence of systemic lúpus erythematosus in a tropical region (Natal, Brazil). Lupus, 11: 528-32.

20. POLESE, J.C. Lúpus Eritematoso sistêmico (LES). In: WIBELINGER, L.M. Fisioterapia em Reumatologia. 1. ed. Rio de Janeiro: Revinter, 2009, cap.07, p. 124-137.

21. VELÁZQUEZ, C.R. Lúpus Eritematoso Sistêmico. In: LATINIS, K.M. Reumatologia. 1.ed. Rio de Janeiro: Guanabara Koogan, 2005.

22. MARCK, H.B. Manual Merck: Diagnóstico e Tratamento. 18 ed. São Paulo: Roca, 2008.

23. Phillips CM. Nutrigenetics and metabolic disease: Current status and

implications for personalised nutrition. Nutrients. 2013;5(1):32–57.

24. Albert PR. What is a functional genetic polymorphism? Defining classes

of functionality. Vol. 36, Journal of Psychiatry and Neuroscience.

Canadian Medical Association; 2011. p. 363–5.

25. Fenech M, El-Sohemy A, Cahill L, Ferguson LR, French TAC, Tai ES, et

al. Nutrigenetics and nutrigenomics: Viewpoints on the current status and applications in nutrition research and practice. J Nutrigenet Nutrigenomics. 2011;4(2):69–89.

26. Abdi R., Smith R.N., Makhlouf L., Najafian N., Luster A.D., Auchincloss H., Jr The role of CC chemokine receptor 5 (CCR5) in islet allograft rejection. Diabetes. 2002;51(8):2489–2495.

27. Chamberlain G., Fox J., Ashton B., Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25(11):2739–2749.

28. de Oliveira C.E., Oda J.M., Losi Guembarovski R., de Oliveira K.B., Ariza C.B., Neto J.S. CC chemokine receptor 5: the interface of host immunity and cancer. Dis Markers. 2014;2014

29. Velasco-Velázquez M., Xolalpa W., Pestell R.G. The potential to target CCL5/CCR5 in breast cancer. Expert Opin Ther Targets. 2014;18(11):1265–1275.

30. Singh S.K., Mishra M.K., Eltoum I.A., Bae S., Lillard J.W., Jr, Singh R. CCR5/CCL5 axis interaction promotes migratory and invasiveness of pancreatic cancer cells. Sci Rep. 2018;8(1):1323.

31. Alkhatib G. The biology of CCR5 and CXCR4. Curr Opin HIV AIDS. 2009;4(2):96–103.

32. Lederman M.M., Penn-Nicholson A., Cho M., Mosier D. Biology of CCR5 and its role in HIV infection and treatment. JAMA. 2006;296(7):815–826.

33. Moore J.P., Kitchen S.G., Pugach P., Zack J.A. The CCR5 and CXCR4 coreceptors-central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. AIDS Res Hum Retroviruses. 2004;20(1):111–126.

34. Jones K.L., Maguire J.J., Davenport A.P. Chemokine receptor CCR5: from AIDS to atherosclerosis. Br J Pharmacol. 2011;162(7):1453–1469.

35. Barmania F., Pepper M.S. C-C chemokine receptor type five (CCR5): an emerging target for the control of HIV infection. Appl Transl Genom. 2013;2:3–16.

36. Scurci I., Martins E., Hartley O. CCR5: established paradigms and new frontiers for a ‘celebrity’ chemokine receptor. Cytokine. 2018;109:81–93.

37. Blanpain C., Migeotte I., Lee B., Vakili J., Doranz B.J., Govaerts C., Vassart G., Doms R.W., Parmentier M. CCR5 binds multiple CC-chemokines: MCP-3 acts as a natural antagonist. Blood. 1999;94:1899–1905. doi: 10.1182/blood.V94.6.1899.

38. Zlotnik A., Yoshie O. Chemokines: a new classification system and their role in immunity. Immunity. 2000;12:121–127. doi: 10.1016/s1074-7613(00)80165-x.

39. Glass W.G., Rosenberg H.F., Murphy P.M. Chemokine regulation of inflammation during acute viral infection. Curr. Opin. Allergy Clin. Immunol. 2003;3:467–473. doi: 10.1097/01.all.0000104448.09202.91.

40. Alkhatib G. The biology of CCR5 and CXCR4. Curr. Opin. HIV AIDS. 2009;4:96–103. doi: 10.1097/COH.0b013e328324bbec.

41. Griffith J.W., Sokol C.L., Luster A.D. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659–702.

42. Lederman M.M., Penn-Nicholson A., Cho M., Mosier D. Biology of CCR5 and its role in HIV infection and treatment. JAMA. 2006;296(7):815–826.

43. Wysocki C.A., Jiang Q., Panoskaltsis-Mortari A., Taylor P.A., McKinnon K.P., Su L. Critical role for CCR5 in the function of donor CD4+CD25+ regulatory T cells during acute graft-versus-host disease. Blood. 2005;106(9):3300–3307.

44. Hoover K.C. Intragenus (Homo) variation in a chemokine receptor gene (CCR5) PLoS One. 2018;13 doi: 10.1371/journal.pone.0204989.

45. Venuti A., Pastori C., Siracusano G., Riva A., Sciortino M.T., Lopalco L. ERK1-based pathway as a new selective mechanism to modulate CCR5 with natural antibodies. J. Immunol. 2015;195:3045–3057. doi: 10.4049/jimmunol.1500708.

46. Venuti A., Pastori C., Siracusano G., Pennisi R., Riva A., Tommasino M., Sciortino M.T., Lopalco L. The abrogation of phosphorylation plays a relevant role in the CCR5 signalosome formation with natural antibodies to CCR5. Viruses. 2018;10:9. doi: 10.3390/v10010009.

47. Achour L., Scott M.G., Shirvani H., Thuret A., Bismuth G., Labbé-Jullié C., Marullo S. CD4-CCR5 interaction in intracellular compartments contributes to receptor expression at the cell surface. Blood. 2009;113:1938–1947. doi: 10.1182/blood-2008-02-141275.

48. Solloch U.V., Lang K., Lange V., Böhme I., Schmidt A.H., Sauter J. Frequencies of gene variant CCR5-Δ32 in 87 countries based on next-generation sequencing of 1.3 million individuals sampled from 3 national DKMS donor centers. Hum. Immunol. 2017;78:710–717. doi: 10.1016/j.humimm.2017.10.001.

49. Silva-Carvalho W.H.V., de Moura R.R., Coelho A.V.C., Crovella S., Guimarães R.L. Frequency of the CCR5-delta32 allele in Brazilian populations: a systematic literature review and meta-analysis. Infect. Genet. Evol. 2016;43:101–107. doi: 10.1016/j.meegid.2016.05.024.

50. Boldt A.B.W., Culpi L., Tsuneto L.T., Souza I.R., Kun J.F.J., Petzl-Erler L.M. Analysis of the CCR5 gene coding region diversity in five South American populations reveals two new non-synonymous alleles in Amerindians and high CCR5*D32 frequency in Euro-Brazilians. Genet. Mol. Biol. 2009;32:12–19. doi: 10.1590/S1415-47572009005000011.

51. Pena S.D.J., Di Pietro G., Fuchshuber-Moraes M., Genro J.P., Hutz M.H. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. PLoS One. 2011;6 doi: 10.1371/journal.pone.0017063.

52. Schauren JS, Marasca JA, Veit TD, et al. CCR5delta32 in systemic lupus erythematosus: implications for disease susceptibility and outcome in a Brazilian population. Lupus. 2013;22(8):802-809.

53. Nguyêñ G.T., Carrington M., Beeler J.A., Dean M., Aledort L.M., Blatt P.M. Phenotypic expressions of CCR5-Δ32/Δ32 homozygosity. J Acquir Immune Defic Syndr. 1999;22(1):75–82.

54. Vargas A.E., Cechim G., Correa J.F., Gomes P.A., Macedo G.S., de Medeiros R.M. Pros and cons of a missing chemokine receptor - Comments on “Is the European spatial distribution of the HIV-1-resistant CCR5-Δ32 allele formed by a breakdown of the pathocenosis due to the historical Roman expansion?” by Eric Faure and Manuela Royer-Carenzi (2008) Infect Genet Evol. 2009;9(4):387–389

55. Weidenbusch M, Kulkarni OP, Anders H-J. The innate immune system in human systemic lupus erythematosus. Clin Sc. 2017;131:625–634.

56. Atabati H, Esmaeili SA, Saburi E, Akhlaghi M, Raoofi A, Rezaei N, et al. Probiotics with ameliorating effects on the severity of skin inflammation in psoriasis: Evidence from experimental and clinical studies. J Cell Physiol. 2020;235:8925–8937.

57. Audiger C, Rahman MJ, Yun TJ, Tarbell KV, Lesage S. The importance of dendritic cells in maintaining immune tolerance. J Immunol. 2017;198:2223–2231.

58. Radmanesh F, Mahmoudi M, Yazdanpanah E, Keyvani V, Kia N, Nikpoor AR, et al. The immunomodulatory effects of mesenchymal stromal cell-based therapy in human and animal models of systemic lupus erythematosus. IUBMB life. 2020;72:2366–2381.

59. Turner J-E, Paust H-J, Bennstein SB, Bramke P, Krebs C, Steinmetz OM, et al. Protective role for CCR5 in murine lupus nephritis. Am J Physiol Renal Physiol. 2012;302:F1503–F1515.

60. Bignon A, Gaudin F, Hémon P, Tharinger H, Mayol K, Walzer T, et al. CCR1 inhibition ameliorates the progression of lupus nephritis in NZB/W mice. J Immunol. 2014;192:886–896.

61. Aguilar, F., Núñez-Roldán, A., Torres, B., Wichmann, I., Sánchez-Román, J., González-Escribano, M.F. 2003. Chemokine receptor CCR2/CCR5 polymorphism in Spanish patients with systemic lupus erythematosus. The Journal of rheumatology, 30: 1770-4.

62. Deng H., Liu R., Ellmeier W., Choe S., Unutmaz D., Burkhart M., Di Marzio P., Marmon S., Sutton R.E., Hill C.M., Davis C.B., Peiper S.C., Schall T.J., Littman D.R., Landau N.R. Identification of a major co-receptor for primary isolates of HIV-1. Nature. 1996;381:661–666. doi: 10.1038/381661a0.

63. Dragic T., Litwin V., Allaway G.P., Martin S.R., Huang Y., Nagashima K.A., Cayanan C., Maddon P.J., Koup R.A., Moore J.P., Paxton W.A. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. Nature. 1996;381:667–673. doi: 10.1038/381667a0.

64. Huang Y., Paxton W.A., Wolinsky S.M., Neumann A.U., Zhang L., He T., Kang S., Ceradini D., Jin Z., Yazdanbakhsh K., Kunstman K., Erickson D., Dragon E., Landau N.R., Phair J., Ho D.D., Koup R.A. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat. Med. 1996;2:1240–1243. doi: 10.1038/nm1196-1240.

65. Samson M., Libert F., Doranz B.J., Rucker J., Liesnard C., Farber C.M., Saragosti S., Lapouméroulie C., Cognaux J., Forceille C., Muyldermans G., Verhofstede C., Burtonboy G., Georges M., Imai T., Rana S., Yi Y., Smyth R.J., Collman R.G., Doms R.W., Vassart G., Parmentier M. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature. 1996;382:722–725. doi: 10.1038/382722a0.

66. Wu L., Paxton W.A., Kassam N., Ruffing N., Rottman J.B., Sullivan N., Choe H., Sodroski J., Newman W., Koup R.A., Mackay C.R. CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, in vitro. J. Exp. Med. 1997;185:1681–1691. doi: 10.1084/jem.185.9.1681.

67. Proudfoot A.E. Chemokine receptors: multifaceted therapeutic targets. Nat. Rev. Immunol. 2002;2:106–115. doi: 10.1038/nri722.

68. Venkatesan S., Petrovic A., Van Ryk D.I., Locati M., Weissman D., Murphy P.M. Reduced cell surface expression of CCR5 in CCR5Δ32 heterozygotes is mediated by gene dosage, rather than by receptor sequestration. J. Biol. Chem. 2002;277:2287–2301. doi: 10.1074/jbc.M108321200.

69. Picton A.C.P., Shalekoff S., Paximadis M., Tiemessen C.T. Marked differences in CCR5 expression and activation levels in two South African populations. Immunology. 2012;136:397–407. doi: 10.1111/j.1365-2567.2012.03592.x.

70. Baltus THL, Kallaur AP, Lozovoy MAB, Morimoto HK, Delongui F, Alfieri DF, et al.. Ccr5Δ32 (Rs333) Polymorphism is Associated With the Susceptibility to Systemic Lupus Erythematosus in Female Brazilian Patients. Rheumatol Int (2016) 36:7–15. doi: 10.1007/s00296-015-3308-z

71. Balasubramanian SP, Cox A, Brown NJ, Reed MW. Candidate gene polymorphisms in solid cancers. Eur J Surg Oncol. 2004;30(6):593-601. doi:10.1016/j.ejso.2004.04.001

72. Menezes CA, Alves Junior ER, Costa GN de O, Dombroski TCD, Mattos RT de, Gomes J de AS, et al.. Genetic polymorphisms and plasma concentrations of leptin (rs7799039) and adiponectin (rs17300539) are associated with obesity in children and adolescents. Rev paul pediatr [Internet]. 2022;40(Rev. paul. pediatr., 2022 40). Available from: https://doi.org/10.1590/1984-0462/2022/40/2021030IN

73. Assakawa, Samantha & Oliveira, Roque & Pimenta, Raphael. (2019). A NUTRIGENÔMICA COMO MÉTODO DE PREVENÇÃO E TRATAMENTO DE DOENÇAS Nutrigenomic as a method of prevention and treatment of diseases La nutrigenomica como método de prevención y tratamiento de enfermedad. 10.20873/uftv6-7029.

74. Kulmann-Leal B, Ellwanger JH, Chies JAB. CCR5Δ32 in Brazil: Impacts of a European Genetic Variant on a Highly Admixed Population. Front Immunol. 2021 Dec 10;12:758358. doi: 10.3389/fimmu.2021.758358. PMID: 34956188; PMCID: PMC8703165.

75. Palomino, Diana Carolina Torres, & Marti, Luciana Cavalheiro. (2015). Quimiocinas e imunidade. einstein (São Paulo), 13(3), 469-473. https://doi.org/10.1590/S1679-45082015RB3438